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THE MAKING OF A PARASITOLOGIST*

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Twenty years ago a group of parasitologists in Washington and Baltimore, where parasitologists were largely concentrated in those days, felt that interest of zoologists, veterinarians, and medical and public health men in parasitology had so greatly increased in the last few years that there was a definite demand for a society that would bring together all the varied individuals who had an interest in this subject. A committee consisting of Dr. R. W. Hegner, Dr. Eloise B. Cram, Dr. B. H. Ransom and Dr. W. W. Cort called a meeting and organized the American Society of Parasitologists, with the late-lamented Dr. H. B. Ward as its first president, Dr. S. T. Darling as its vice president, and Dr. W. W. Cort as its secretary and treasurer.

That occasion, ladies and gentlemen, marked the weaning of parasitology in the United States. For the first time it was permitted to stand on its own feet as a separate scientific entity, and to gather its own nourishment on which to grow and develop. For many years thereafter it was still considered a child, and as Dr. M. C. Hall pointed out in his presidential address in 1932, there were even some who questioned its right to be called a science; yet its growth continued steadily, and its health was always good.

It was not until the outbreak of the recent global war that parasitology took its rightful place of importance in the community of sciences, and was given the recognition to which it had a rightful claim. Today, my friends, parasitology in America has come of age.

When the recent world-wide conflict developed, the need for parasitology became painfully apparent, and the neglect with which it had been treated in the past became a very serious matter. All over the world parasitological problems presented themselves for immediate solution—malaria contributed no small part to our defeat at Bataan, and it made it questionable whether our troops could fight successfully in New Guinea, although military necessity made it imperative. On the far-flung military fronts of the Pacific, Asia, Europe, Africa and Tropical America our troops were beset by, or threatened with, malaria, amebiasis, leishmaniasis, trypanosomiasis, schistosomiasis, filariasis, and a host of parasite-borne diseases, such as typhus, tsutsugamushi, relapsing fever, yellow fever, dengue, encephalomyelitis, and plague. Yet in the medical corps of our army and navy there was a mere handful of men who had had experience with even such common parasitic diseases as malaria or amebiasis; most of them had never heard of schistosomiasis or leishmaniasis, or if they had, it was years before when some teacher in a medical school casually mentioned them in

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a lecture that they were peacefully slumbering through. One of my good friends who was in charge of a large hospital for returning veterans told me that of the twenty physicians working in that hospital, not one had ever seen a case of malaria before his assignment there, yet malaria was the commonest infection among the patients dealth with.

Our armed forces recognized the dilemma and performed a veritable miracle in correcting it. Teachers in bacteriology departments in our medical schools were sent where they could be given special courses in parasitology; parasitological training was given to medical officers in the service; a parasitological distribution center was set up by the army to provide specimens for schools all over the country. The National Research Council sponsored research projects to acquire new and urgently needed information, and commissions were organized and sent to all corners of the world to investigate special problems as they arose, or to develop adequate control or protective measures. The membership of this society participated in this feverish activity to a very remarkable extent; its contribution to the war effort, quantitatively and qualitatively, was something of which we can be very proud.

So, friends, I say that today we can celebrate the coming of age of parasitology in America. But with maturity comes additional tasks and additional responsibility. Our importance to the country did not end with the war. Not only will there be an unprecedented number of imported cases of parasitic disease in returning military personnel, with the possibility of the establishment of endemic foci in some cases, but social and economic relations with other parts of the world have brought us to a point where we can no longer take a merely academic interest in diseases of other countries, and particularly those of Tropical America and the Far East. As far as parasitology is concerned isolationism is gone forever, and we are citizens of One World, a world that has shrunken to a point where it is not more than 60 hours between any two capitals on the globe.

It is not necessary to point out to this group what this may mean in the opportunities afforded for the introduction and spread of disease and disease vectors if we do not guard against it. Even twenty years ago we did not need to worry much about the importation of tsetse flies from Africa, or scrub typhus-infected red bugs from New Guinea, or virus-infected ticks from Russia. Today there are few diseases and few disease vectors from which we can consider ourselves entirely safe. Our only protection is eternal vigilance, and to make our vigilance effective we must be well informed, and cognizant of the existence in nature of direct or indirect threats from outside.

In the first presidential address before this society twenty years ago, Dr. H. B. Ward justified the establishment of the Society on the basis of the rapid growth of knowledge in the field, most of it within the present century; on the increasing number of workers in the field; and on the continuing future opportunity and need. Dr. Ward lived long enough to see these opportunities and needs develop to a degree that must have been a revelation even to him.

With the tremendous outburst of interest in parasitology that was engendered by the demands of the war, there has been a great increase in the membership of our society, and many individuals are now anxious to be recognized as parasitologists who formerly would have been content to acknowledge a passing interest in it. It is time, therefore, since there will be more people aspiring to become parasitologists

than ever before, and many who will wish to reconvert to this now popular field, that we stop and consider what *is* a parasitologist and what it takes to make one.

In the first place, since Dr. Hall in a presidential address before this society in 1932 established in his inimitably brilliant manner the fact that parasitology is a science, it follows without question that a parasitologist is a scientist, or should be. As such he must have certain attributes that are common to all scientists, attributes that are inherent in him, and which no amount of training or experience will be able to bring out if they are not there to start with. Perhaps, therefore, it would have been a better choice of words for the title of this address if I had called it "The Development of a Parasitologist" rather than "The Making of a Parasitologist."

As Hall pointed out, the dictionary defines science as classified knowledge, but as usual the dictionary turns out to be uninspired and superficial, for science is a dynamic, not a static thing. It involves hunting for new facts, derivation of sound conclusions from these facts, and application of the conclusions to new problems. A scientist must be keen of intellect, sound in judgment, observing, imaginative, persevering, industrious, and endowed with more than a normal amount of initiative and resourcefulness. He must also be imbued with courage, honesty, and an impersonal love of truth.

In addition to these general requirements of scientists, the parasitologist must have, as Hall remarked, a large capacity for hard and dirty work, with particular emphasis on the dirt. A parasitologist must inevitably, if he is not exclusively a malariologist or a chemotherapist, spend many uncomfortable hours performing autopsies on animals that are not always freshly dead, in wallowing in blood or intestinal contents, in spending whole afternoons with the excreta of man or animals, or in tramping through swamps and jungles in search of snails or insects. A man or woman who hates bad smells, avoids blood and filth, or shudders at dirty hands, cannot be a full-fledged parasitologist. With these basic qualifications, a parasitologist can be developed, although it might be easier to develop almost any other kind of scientist out of him, because probably no other branch of natural science dovetails into, or overlaps, so many other branches.

The prime necessity for any scientist, and for parasitologists as much as any, is familiarity with the literature, for proper utilization of which he should have a reading knowledge of German, French and Latin. With these he should be able, with the help of a dictionary, a simple grammar, and a few headaches, to get the essential facts from papers written in Italian, Spanish or Portuguese. With the development of interest in parasitology in Latin-American countries, and with the ever-closer ties between all the American republics, ability to make use of Spanish and Portuguese is becoming more and more necessary. Literature in slavic and oriental languages, particularly Russian, is also assuming a more and more prominent place, but it is to be hoped that authors publishing their work in languages that are not of Latin, Germanic or Anglo-Saxon origin will be co-operative to the extent of publishing abstracts in one of these languages.

It is, of course, important to be able to track down papers dealing with a subject as well as to be able to read them, so a parasitologist must be familiar with the various bibliographic aids. It sometimes takes a veritable Sherlock Holmes to ferret out all the pertinent literature, and it takes experience. There is probably no one of us who has not been chagrined at having overlooked references that would have

been helpful or would have saved us from the suspicion of carelessly having failed to give credit where credit was due.

Another qualification that every parasitologist should have is a knowledge of the English language, especially spelling and grammar. I can perhaps speak more feelingly than most of you on this subject, except those who are or have been editors of journals, because of my experience as editor of the parasitology section of Biological Abstracts. Of course, knowledge of spelling and grammar is not all that is necessary. For proper presentation of one's work one must know how to separate the wheat from the chaff, and also to avoid using 100 words where ten would do better. When I was a graduate student I was told that if I had something to say I would be able to say it, and was not encouraged to take a course in writing. With this I do not agree; granted that the first essential is having something to say, it is almost as important to be able to say it in such a way as to satisfy an editor whose space is at a premium, and to satisfy a reader who may not be a mind reader. For strictly technical papers it isn't necessary to hold a reader's attention by flowery, clever or appealing use of words or phrases, but most parasitologists will sooner or later find it desirable or expedient to popularize his work in one way or another, if not for the so-called man in the street, so he can answer a $4.00 question, at least to develop interest in students, or to influence legislators, industrialists or philanthropists who may be as important to research as the research man himself. Parasitologists need money, and not many of them have it themselves, so they must interest those who do.

Now let me go back to the parasitologists' relation to other sciences. I will not have to spend much time on the need for a basic knowledge of chemistry, physics, and mathematics. The chemistry is involved in pharmacology and therapeutics, preparation of culture media, staining techniques, biochemical reactions, and in so many other ways that it would be difficult to find any parasitological problem that would not demand some chemical knowledge early in its elucidation. Physics is needed in connection with microscopy; the effects of light, heat, and moisture; the effects of surface tension; and the action of various electro-magnetic rays, to mention some of the most obvious applications. Mathematics is perhaps least appreciated and most abhorred by parasitologists as a rule, but much work could be improved, and many invalid conclusions avoided, if the parasitologist at least understood logarithms, could solve simple differential equations, and understood statistical methods.

In the biological sciences I believe that the parasitologist has a wider range of interests, and has to stick his nose, literally and figuratively, into more things than any other kind of a biologist. The biological sciences may be subdivided either phylogenetically, from protozoology to mammalogy, or subjectively into such subjects as morphology, histology, cytology, physiology, embryology, pathology, immunology, ecology, genetics, evolution, etc. Each one of the taxonomic divisions cuts across the various subjective divisions, and vice versa. But where does parasitology fit in? It burrows, to a greater or lesser extent, into every division in both categories.

In the first place, parasitism has developed in practically every animal phylum, and any vertebrate host species studied is almost sure to be subject to parasitism by representatives of at least four different phyla. Certainly a parasitologist should have some familiarity with the taxonomy and classification of Protozoa, flatworms, nematodes, annelids and arthropods.

In the second place, there is probably no species of metazoan animal that is not subject to parasitism. A medical parasitologist may not have much interest in the parasitic tribulations of a woodpecker or a starfish, but he will sooner or later have to be concerned with those of molluscs, crustaceans, arachnids, insects, fishes, and many other kinds of birds and mammals, and even occasionally amphibians and reptiles, for members of all these groups serve either as intermediate hosts or reservoir hosts for human parasites. The veterinary parasitologist will not be concerned alone with definitive hosts ranging from canaries to horses, but will be concerned with an equally long list of intermediate or reservoir hosts. If interested in the parasites of pigs, for instance, he would have to know something about parasitism in annelids, molluscs, crustaceans, arachnids, insects, rats, man, and even armadillos, for in our laboratory we have recently found that armadillos serve more or less the function of vacuum sweepers for hog parasites that undergo their early development in grubs and other arthropods. The parasitologist who concerns himself with parasites of wild game or fish must have an even broader knowledge of zoology.

Not only must the parasitologist, if he is prepared to undertake all phases of a problem, be concerned with parasitism in these animals, but he must know something about the animals themselves—their anatomy, their life cycles, their habits, and their ecology. Dr. Hall says that he found a group of university students who had never thought of parasitology as dealing with anything more than the morphology and life cycles of parasites. He relates that soon after he entered the Bureau of Animal Industry Dr. Curtice asked him if he was only interested in parasites, or also in the dear horse, the dear cow and the dear sheep. When he replied that he aimed to be a zoologist and was interested in worms and not in livestock, Dr. Curtice informed him that he belonged in a museum, not in the Bureau of Animal Industry.

A parasitologist must be familiar with the natural and artificial habitats of his host animals, and so may need to know something about farm and range practices, wild life management, fish culture, and the like. Only by careful study of these things can adequate methods of control of parasitic infections be worked out. The first step in developing control measures is to find the weakest link in the chain of life cycle events, and only a thorough knowledge of environmental conditions as well as parasite and host physiology will make this possible.

Some knowledge of geography and geographical distribution is also desirable, with appreciation of such things as zoological regions, life zones, biotas, and lesser ecological units. In my experience geography is a sadly neglected subject in high schools, and is usually neglected altogether in college. I have found students in a sophomore class prior to the war who thought that Algeria was in Canada and the Philippine Islands in the West Indies. The war has probably helped this situation to some extent, but not enough.

Not only do parasites belong to a number of different phyla, and their hosts to all of them, but in addition there is no organ or tissue that is not subject to attack, and hardly a pathological condition, if in fact any, that cannot be produced. Therefore a parasitologist should know something about the anatomy, histology, physiology, pathology, and ecology of the hosts. Embryology and development, also, are frequently involved, as in the case of hereditary transmission of parasites in arthropods, penetration of fetal membranes by parasites, and embryonic cultivation. Lack

of knowledge of nutrition would often leave a parasitologist without important clues he needs. I need only mention the effect of diet on intestinal flagellates, the influence of diet on hookworm disease, and the relation of tapeworms to vitamins and the possible clue it gives to *Diphyllobothrium* anemia and the systemic effects of tapeworm infections.

The importance of ecology is concerned not only with the life cycles and epidemiology of parasitic infections, but also with their pathological effects. Dr. Strong has ably called attention to the complexity of the problem of mutual relations between organisms and their environment as it affects pathological processes. Climate and meteorological conditions are fundamental determining factors with respect to the flora and fauna of a region, influencing intermediate and reservoir hosts. They also have profound effects on resistance to disease, as has been ably demonstrated by Strong, Graham, Reed, and others. Adaptation of the white man to the tropics involves far more than the direct effects of heat and sun. The effect of climate as a predisposing agent to disease is only beginning to be understood; that the effect is important is apparent from the distribution of such diseases as yaws, pinta, sprue, dysenteries, Chagas' disease, cholera, and respiratory infections, in all of which it is probable that the effects of climate are due to influences on the host as well as on the parasites. Experience in the recent war brought this aspect of disease so much into the limelight that Col. Dieuaide suggested the need of a distinctive name for it, geopathology.

The importance of heredity in parasitology is only beginning to be understood. We all know that for every patent case of poliomyelitis, meningitis, or tuberculosis there are several times, sometimes possibly tens of times, as many inapparent cases. Heredity is certainly only one of the factors involved, but it is an important one. Huff has shown the influence of hereditary susceptibility of *Culex* mosquitoes to bird malaria, Becker of rats to coccidiosis, Curtis Dunning and Bullock of rats to cysticercus sarcoma, and Warwick of rats to *Nippostrongylus* infections, to mention only a few. It is a subject that has scarcely been scratched, but may develop into an important field in the future. Certainly a parasitologist should be prepared for observations in this field by a knowledge of the fundamentals of genetics.

Immunology affects parasitism in so many ways that no parasitologist should be ignorant of its essentials. Immunology in parasitic infections is perhaps the most promising direction for future research in the entire field. Although immunity to various parasitic infections had been observed, and a number of immunological diagnostic tests developed during the early part of this century, the first important studies on its mechanism were made by W. H. and L. C. Taliaferro only 20 years ago, and the publication of Taliaferro's book on immunology of parasitic infections in 1929 set a landmark. Since then numerous contributions by many parasitologists have been made, but it is still only a beginning.

Immunology, as I said, concerns parasitology in many ways. It is concerned with development of resistance, with modification of pathological effects, with techniques, and with the understanding of pathological processes, particularly in connection with sensitization and allergy. Sensitization and allergy have been shown to be of profound importance, for example, in filariasis, scabies, pediculosis, and even flea bites. Diagnostic procedures include complement fixation, agglutination, precipitation, and skin tests. Little is yet known about immunological reactions on the part

of parasites or their vectors, and the review of the matter made by Huff a few years ago was not very encouraging for work along this line, although adaptational changes certainly do occur, as in the development of drug fastness in Protozoa.

Parasitologists need some knowledge of bacteriology and virology. Many parasitic infections are profoundly affected by the concurrent presence of bacteria, as for example in amebiasis, leishmaniasis and filariasis. In a number of instances helminth parasites have been shown to be vectors of viruses, and lesions produced by helminths are widely believed to act as portals of entry for bacteria. Knowledge of bacteriological technique is essential in connection with artificial cultivation of parasites, and development of aseptic methods. Concomitant bacterial and parasitic infections frequently present problems in therapy that involve careful timing and the weighing of probabilities and values. As the writer said in a recent paper, infectious disease is an extraordinarily complicated phenomenon. The interaction of a parasite and its host is not a static thing like the interaction of one chemical with another, capable of simple description, and following a well-defined course. Even if we know what organism causes a disease, and something about its biology, we may be far from understanding the disease it causes. We are dealing with the interaction of two organisms both of which are capable of continuous adaptation to changing conditions. A disease, like an organism, is born, it grows, it adapts itself to environment, and it finally dies. During its life it is influenced by a host of environmental factors which may profoundly alter its development.

A parasitologist must also be familiar with his tools. He must be expert in the use of the microscope, and of microscopic technique. He should understand the use of sterilization methods, incubators, water baths, constant temperature and humidity apparatus, inoculation techniques, and methods for artificial cultivation of both micro-organisms and invertebrate animals. Often these have to be devised from methods applicable to related or analogous organisms. In fact there are very few parasitological investigations, even of the caliber of Ph.D. dissertations, that do not involve the modification of old techniques or development of new ones. Therefore what is needed is not a mere ability to follow directions in a book, but the understanding of underlying principles. The former can be acquired by experience, the latter only by education. A parasitologist should also know something about photography, and should have had some training and experience in making drawings.

Even more fundamental is a knowledge of research methods in general—such as the proper balancing of irrelevant factors while varying the experimental one, the correct use of checks and controls, the proper evaluation of experimental error, and the use of caution and conservatism in the drawing of conclusions.

Now, if proficiency in all the things I have mentioned were required before a person could call himself a parasitologist, and only parasitologists were permitted at this gathering, I suspect that there would be an extremely small audience, and certainly no speaker, unless a substitute were found for the one you are presently bearing with. No one, even after he has become a sufficiently renowned parasitologist to be an officer or councillor of this august society, can be expected to be thoroughly grounded in all the things I have mentioned, much less a beginner. But I do believe that anyone who can be called a parasitologist should be thoroughly grounded in some of them, and have had at least an introduction to most of them. I should, perhaps, remark at this point that what I mean by a parasitologist is a scientist who can under-

take the solution of at least a considerable variety of parasitological problems, and follow up the various lines of research that are sure to open up whenever a problem is attacked.

If you agree with me that at least a bowing acquaintance with the various fields of knowledge mentioned above are necessary for a parasitologist, you will also agree, I am sure, with the contention that any formal education less than that equivalent to the requirements for a Ph.D. degree would be very likely to fall short of the mark. Experience will develop proficiency in almost all of the fields of knowledge I have mentioned, but it will rarely if ever provide the broad, well-rounded training that education provides. To grow well and mature properly a plant needs good roots, or else it will soon reach the limit of its capacity for growth. For a parasitologist, education supplies the roots and stems, experience the foliage, flowers and fruit.

I mention this particularly because the U. S. Civil Service Commission has recently been inclined to substitute experience for education, especially in the case of veterans applying for professional jobs. In considering a minimum educational requirement and deciding for a given position that the duties "cannot be performed by a person who does not have such education" the Commission must be convinced that persons who lack the educational training could not perform the duties successfully. So far the Commission has been inclined to stick to its educational requirements only for cases like physicians or veterinarians whose training is prescribed by law. In a letter to the writer dated Sept. 26, 1945, the Commission stated that it had determined that the duties of Agronomists, Soil Scientists and Range Conservationists in the field of biological science demand the minimum educational requirements prescribed by the Commission. It is to be hoped that after further discussions that were promised, parasitologists will be included in this list. In a letter to the Civil Service Commission last August, written in behalf of the Officers and Councillors of this Society, I pointed out to them that a professional parasitologist, to be able to carry on research in his field, requires most of the training of a physician or a veterinarian and much more in addition.

A questionnaire was sent to the officers and councillors of our society in which their opinion was asked as to what percentage of parasitologists had come up without college training, and what percentage with a bachelor's, master's, or doctor's degree respectively. The answers were strikingly uniform. All agreed that practically none had come up without college education. The general opinion was that about 5% of parasitologists had only a bachelor's degree, 10% only a master's, and 85% had a doctorate. The roster of members of this society, including 736 names, included 2.7% with no degrees listed, 12% with only a bachelor's degree, 19.3% with only a master's degree, and 65% with a doctor's degree. Since the society may admit to membership any individual who has shown an interest in parasitology, a large percentage of those without advanced degrees were or are working for advanced degrees, or are individuals who fall into the class of technicians, or professionals in other fields, with a more or less incidental interest. I judge, therefore, that the Society's roster of membership would fully bear out the 5%, 10%, and 85% of parasitologists with a bachelor's, master's, and doctor's degrees, respectively.

It is not possible for a student in his undergraduate course to get more than a foundation in English, foreign languages, and the basic sciences, with a single course in parasitology, and perhaps a course in bacteriology and immunology, or entomol-

ogy. By the time he has obtained the master's degree he should have had additional courses in fundamental biological sciences, some advanced work in some field of parasitology, experience in looking up literature, and a taste of research involving experience with laboratory technique and general research methods. Only by the time he has obtained the Ph.D. degree is he likely to have had enough education and training in his own and related scientific fields, and enough supervised experience in the delineation, accomplishment, and writing up of a research project, so he will be reasonably prepared to undertake investigations of his own. In some favorable situations, where he can have the benefit of guidance and help, he may be able to get by with only a master's degree, but as past evidence clearly shows, this happens in only a small minority of cases.

In conclusion I might compare a parasitologist to an orchid. He requires long and careful nurturing, he develops slowly, and he is himself a parasite in that he is dependent on many other sciences for material aid. But when he comes to flower he is a rare and beautiful object, scientifically speaking, and is usually slow in going to seed. He may not always smell like an orchid, but we can't have everything.

PSEUDOPARASITISM OF THE HUMAN INTESTINAL TRACT

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For the past ten years we have been examining about two hundred samples of human stools annually for protozoal parasites. The specimens were collected in a psychiatric ward; suitable material was used for a course of instruction in tropical medicine. The third of May, 1944, in four out of thirteen specimens, structures were found resembling cysts, of a type never seen in human stools before, neither in Holland nor in Java. Afterwards we never saw them again, either in specimens from other patients or in those of the patients who had produced them on the third of May.

On closer examination and after a study of the literature, they were found to be very much like young oöcysts of *Eimeria debliecki,* the intestinal coccidium of the pig. They are figured in text-figure I.

Text-figure I.

When suspended in a two per cent eosine solution, all oöcysts in the freshly emitted feces stained red, the inner contents as well as the oöcyst walls; apparently all were dead. As was to be expected, after some days, no development of the oöcysts' contents could be traced. Most probably we had to deal with oöcysts ingested with food and merely passing through the intestinal tract, killed either by the preparation of the food or during the passage through the human bowel. Most of the oöcysts were oval in shape (Figs. 1–4, 8, 9); rarely their outline was circular

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(Figs. 11, 12). Usually both the poles of the oval showed the same shape, but some oöcysts were more or less egg-shaped (Figs. 5, 6). Inside the oöcysts was an oval mass, nearly wholly filling the cyst. In some cysts this mass was retracted somewhat from the walls (Figs. 6, 7, 9). Possibly this was an initial stage of development. The inner mass was usually rather homogeneously granular (Figs. 4, 6), sometimes with a clear center, but often showing one or more strongly refringent corpuscles (Figs. 1, 4, 7, 8, 10).

Two hundred oöcysts were measured. The length varied from 18 to 32 μ, being 26.2 μ in the mean; for the breadth these figures were: range, 16 to 24 μ, mean, 19.9 μ. The ratio $\frac{\text{breadth}}{\text{length}}$ varied between 0.59 and 1, the mean being 0.76. These figures tally rather well with those given in Dutch literature for *Eimeria debliecki:* Krediet (1921): length 12–35 μ, breadth 12–24 μ; Munnik (1924): 14–35 and 12–29 μ, means, 24 and 18 μ, mean index 0.75; De Graaf (1925): 13–34 μ and 11–25 μ, mean, 20.7 μ and 15.7 μ, index 0.50–1, mean, 0.763. However, Biester and Murray (1929) stated that "size of oöcysts recovered does not appear to be a sound basis for the differentiation or identification of coccidial species." The morphological resemblance between the oöcysts seen by us and the figures of the authors cited above is most striking.

In order to trace the source of the oöcysts the fares in the psychiatric ward for some days before the third of May were studied. The only suspect food from which coccidia might have originated was liver sausage. Possibly pigs' bowels were used for its preparation.

In the literature, some cases have been recorded of the finding of *Eimeria* oöcysts ingested with food and discharged with the stools without giving rise to an infection of man and simply passing through the intestinal tract without being digested. *Eimeria wenyoni,* found by Wenyon (1916) and named by Dobell (1919), who considered it to be a new species and a true parasite of man, afterwards proved to be neither, being simply *E. clupearum* Thélohan, 1892, a parasite of the liver of herrings, sprats and mackerel, the oöcysts of which had passed through the human intestine (Thomson and Robertson, 1926). The same holds good for *E. oxyspora* and *E. snijdersi* (the latter first seen by Snijders (1921)), thus named by Dobell (1919 and 1921) and also described by this author as true human parasites. In 1922 Thomson and Robertson and in the same year Broughton Alcock and Thomson described the finding of *E. oxyspora* in human stools. Thomson and Robertson (1926) showed them to be *E. sardinae* Thélohan, 1890, parasites of the testis of sprats and herrings and able to pass through the intestine of man (same authors, 1926a).

Wolff (1933) records the findings of *Eimeria* oöcysts in human stools. He is astonished at the scarcity of similar records in the literature, as fish is a common food everywhere and coccidial infection in fish very frequent. Krijgsman (1933) explains this scarcity by the digestion of the otherwise very resistant coccidial oöcyst wall by the intestinal juices. Only in cases of intestinal trouble and digestional disturbance may the oöcysts appear undamaged in the stools. This happens too if by the use of a laxative the oöcysts are exposed for a short time only to the intestinal juices.

SUMMARY

On one day, in four out of thirteen fecal samples from a psychiatric ward coccidial oöcysts, probably of *Eimeria debliecki,* were found. The cysts stained red with eosine solution, and were apparently dead. Possibly they had been ingested with liver sausage for the preparation of which pigs' bowels may have been used.

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STUDIES ON *TRICHOMONAS GALLINAE* INFECTIONS IN PIGEON SQUABS*

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INTRODUCTION

A survey of the literature shows that *Trichomonas gallinae,* pathogenic for birds, has been experimentally placed in a variety of avian hosts (see review paper by Levine, Boley, and Hester, 1941). To mention only a few, natural occurrences of trichomoniasis have been reported in domestic varieties of turkeys (Volkmar, 1930; Hawn, 1937), chickens (Levine and Brandly, 1939), and pigeons (Waller, 1934; Cauthen, 1936; Hurt, 1945). Considerable experimental use has been made of these hosts. Cultures, presumably with bacteria, have been placed in the mouth and throat with (Hawn, 1937) and without (Levine, Boley, and Hester, 1941) superficial scarification. Cultures from, or emulsions of, infected tissues have been both fed to certain of these birds (Bos, 1933, 1934; Waller, 1934; Hawn, 1937) and variously injected into their livers, oral mucosas, and skins (Bos, 1933, 1934; Waller, 1934; Cauthen, 1936).

Two drawbacks are to be found in much of the work with *T. gallinae.* First, the inocula were all too frequently contaminated with bacteria, so that the true rôle of the various agents in the production of the disturbances could not be determined. Exceptions are Bos (1933, 1934) and Cauthen (1936), who used pure cultures of *T. gallinae.* Secondly, information is generally lacking as to whether or not the experimental hosts were free of this *Trichomonas* before the experiments were performed. This is not so serious when one is dealing with passerines such as starlings and English sparrows (Levine, Boley, and Hester, 1941) or gallinaceous birds such as quail, chickens, turkeys, and pheasants (Levine, Boley, and Hester, 1941; Levine and Brandly, 1940; Cauthen, 1936; Hawn, 1937). In them the fulminating trichomoniasis is the rule, and the host-parasite relations preclude much transfer of the flagellate from one generation to the next.

In columbid birds (pigeons and doves) on the other hand, the establishment of freedom from this *Trichomonas* before infection is of paramount importance. This is because of the high incidence of *T. gallinae* in the general population, and because of the regurgitational type of feeding practiced by these birds in which the squab is probably infected at the very first meal with whatever organism is present in the crop of its parent.

Bos (1933, 1934), Waller (1934), and Cauthen (1936) have done considerable work on pigeons, but they include no statement as to whether their experimental birds were or were not naturally infected with *T. gallinae.* Except for certain feeding experiments performed by Waller (1934), these investigators employed the injection method of infection, inserting the trichomonads primarily into the liver, breast muscle, skin, and oral submucosa. One wonders whether or not the injec-

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tion technique was forced by the inability to obtain experimental squabs from stock free from *T. gallinae.*

The results reported here were obtained from the oral implantation of various cultures of *T. gallinae,* and of bacteria, into homing pigeons (*Columba livia*) derived from a loft completely free of *Trichomonas.*

MATERIAL AND METHODS

In 1940 the senior author secured a large series of these pigeons. By careful selection there were established four pairs of breeding birds completely free of *T. gallinae.* From them there developed the negative loft which supplied the clean birds used here. Their ages at experimentation ranged from one to six months, the majority being about seven weeks old.

The strain of *T. gallinae* employed was isolated from the lesions of a peregrine falcon (*Falco peregrinus anatum*) which died of the infection. The cultures made directly from the falcon contained not only the *Trichomonas,* but *Escherichia coli communis, Micrococcus candicans,* and *Bacillus subtilis* as well. These bacteria were not found occurring naturally in the oral cavities of the experimental pigeons. The cultures were maintained indefinitely in modified Ringer's to which a small amount of Loeffler's dried beef serum was added. The bacteria-free cultures of *T. gallinae* were isolated from bacteria-free liver lesions in squabs also infected with the falcon strain of *T. gallinae,* and were maintained on a modified Boeck-Drbohlav medium. All were incubated at about 35° C., and transferred at 48–72-hour intervals. Likewise maintained were cultures of *E. coli communis* and *M. candicans* isolated directly from the falcon's lesions, as well as strains of these two organisms plus *B. subtilis,* which were growing in the original isolations of *T. gallinae* from the same source.

From the above cultures five types of inocula were prepared as follows: 1) the organisms in the cultures as originally isolated from the falcon (i.e., *T. gallinae, E. coli communis, M. candicans,* and *B. subtilis*) ; 2) the above three bacteria alone; 3) the bacteria isolated from the falcon's lesions (i.e., *E. coli communis* and *M. candicans*) ; 4) a combination of types 1) and 3) ; and 5) bacteria-free cultures of *T. gallinae.* One-cc suspensions of these inocula were dropped by pipette at the backs of the pigeons' open mouths. (For an analysis of what constitutes a pigeon's "mouth" and "pharynx" please refer to the Discussion.) In all, forty-two implantations were made; mild oral scarification accompanied the first four. There were no injections.

Attempts were made to demonstrate acid-fast (mycobacteria) and fusospirochaetal organisms in the *Trichomonas* lesions of the falcon. None was found. Cultures for strictly anaerobic organisms were likewise negative.

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OBSERVATIONS

A summary of the implantations and their terminal results are presented in the table.

Implantation. (1) Cultures of *Trichomonas gallinae* plus *E. coli communis, M. candicans,* and *B. subtilis,* as originally isolated from the falcon's lesions. This material was put into the mouths of six squabs. Four died and one recovered, the latter remaining permanently positive for *T. gallinae.* All birds which are reported as having recovered were kept for many weeks in order to be sure that recovery was complete. In fact, some have reached maturity and are serving as positive breeding stock for further experiments. None of the recovered birds which were later posted showed any signs of gross pathology. The sixth was killed for culture

TABLE 1.—*Results of implanting various inocula into the mouths of* Trichomonas gallinae-*free pigeons*

Inoculum	Number of birds	Results
Culture from falcon's mouth containing: *Trichomonas gallinae* ..... *Escherichia coli communis* ..... *Micrococcus candicans* ..... *Bacillus subtilis*	6	4 died from trichomoniasis 1 recovered completely from the early lesions 1 was killed for culture purposes
Bacteria from above	6	No apparent effect
Bacteria direct from falcon's lesions: *E. coli communis* ..... *M. candicans*	6	" " "
Combination of inocula 1 and 3 above	7	2 died from trichomoniasis 4 recovered completely from the early lesions 1 was killed for examination purposes
Trichomonas gallinae bacteria-free	17	15 died from trichomoniasis 1 was killed for culture purposes 1 was killed for demonstration purposes
Totals	42	12 given bacteria only showed no effect 30 received *T. gallinae* ± bacteria. Of these: 4 were killed for various reasons 26 were allowed to run their course. Of these: 5 recovered from their early lesions 21 died from trichomoniasis

purposes. The birds recorded as killed for demonstration or culture purposes all showed lesions typical of the disease. Mild scarification of the oral mucosa was performed on two birds. This appeared to have no influence on the course of the infection or the extent of the lesions. The bacteria in the inocula were not subsequently recoverable from the squabs' mouths and throats.

(2) Cultures of *E. coli communis, M. candicans,* and *B. subtilis* isolated from the foregoing inoculum. These organisms were placed in six squabs. None showed the slightest evidence of disturbance of any kind. On later examination all the implanted bacteria were seen to have disappeared.

(3) *E. coli communis* and *M. candicans* cultured directly from the dead falcon's lesions. Six squabs were used here with completely negative results. Again, the implanted bacteria soon disappeared. Mild scarification in one bird had no apparent influence on the result.

(4) Feeling that the effect of the first inoculum above might be enhanced by the

addition of the bacteria in the third inoculum, these were so mixed and placed in seven squabs. One was killed when it developed a severe lesion in one eye, unrelated to the experimental condition. Two died of trichomoniasis and four recovered. These latter four were used quite late in the study and it is felt that the *Trichomonas* might have become somewhat less virulent than at first. This possibility is to be investigated.

(5) The last and fifth type of inoculum was a pure, bacteria-free culture of *T. gallinae*. This was put into seventeen squabs. Of these, one was killed for class use, another for culture purposes. Fifteen died of trichomoniasis; none recovered. There were no scarifications.

Fom the foregoing experiments it may be seen that pure-culture *Trichomonas gallinae* dropped by pipette into the mouths of young squabs can readily kill these birds. Also, bacteria isolated from the trichomonad lesions of a dead falcon, and bacteria obtained from cultures of *T. gallinae* originally isolated from the same source, failed completely to produce any visible effect. These bacteria soon disappeared from the pigeons' mouths and throats. There is no evidence that *T. gallinae* accompanied by the bacteria from lesions caused by this trichomonad is thereby enhanced in its disease-producing powers.

Of the thirty squabs receiving *T. gallinae,* with and without bacteria, twenty-six were allowed to run the full course of the disease, and four were killed for various reasons. Five of the twenty-six recovered from their initial lesions and became carriers, and twenty-one were killed by the flagellate.

Staphylococcus albus, Neisseria flava, Streptococcus viridans, and *Corynebacterium* sp. were natural residents of the mouths of the experimental squabs. They were present before and after experimentation.

In order to be sure that a virus (pox) was not involved in the death of the falcon from which all the isolations were made, material was sent to Dr. F. R. Beaudette (N. J. Agr. Exp. Sta.), who made egg, comb, and feather follicle inoculations in chickens. All results were negative. The pure cultures of *T. gallinae* were also never used sooner than two weeks after their isolation. It was felt that this was sufficient time to have caused the death in these cultures of all virus which might have come in with the *Trichomonas*.

Gross pathology.—Twelve birds received the bacteria cultures alone. Not the slightest sign of disturbance developed in any one.

Thirty received *T. gallinae*. Every bird here developed a severe, emaciating infection. In the five which recovered, small caseous areas were seen to appear and disappear on the oral, pharyngeal, and upper oesphageal mucosas for weeks after the initial nearly fatal attacks. Thirteen sqaubs received *Trichomonas* and bacteria; seventeen got pure culture *T. gallinae*. There was no evidence that the lesions in these two groups varied in number or extent.

Trichomoniasis, at least with this strain of *T. gallinae,* is a very serious disease. Invariably, the first sign of infection was a small, yellowish area (or areas) which appeared somewhere on the oral mucosa. Usually, there was one initial lesion, which in most cases appeared in the mid-line between the pair of soft palatal flaps located in the pharyngeal region of the mouth. These first lesions appeared 3–14 days (average, 7.4 days) after infection. They generally increased rapidly in size and number, frequently reaching such proportions that the oesophagus or trachea

was blocked completely. The tissues of the roof of the mouth often were extensively invaded, involving the production of large, caseous masses which sometimes even included the bones of the floor of the skull. In one bird these lesions extended to the tissues about the eyes, and trichomonads were recovered in fluid emerging therefrom.

Of all the alimentary tract, the pharyngeal region received the heaviest attack. Here the soft palate, including the flaps to the rear, was frequently a huge mass of caseation which extended forward and to the back in great lumps of yellow necrotic material completely covering all surfaces. The odor from a mouth of this kind was most fetid.

Whereas the oesophagus was occasionally simply lined with caseation, this structure was not so generally involved as the mouth and pharynx. The lesions here frequently presented the appearance of small mounds, from the centers of which extended short spurs. Sometimes there was no gross evidence of disturbance. The crop, too, was commonly free of caseation, but when present the lesions were usually at the base, just anterior to the proventriculus. This latter structure was attacked only on rare occasions, but at least once it was very heavily involved. The gizzard and the small and large intestines were never seen to bear lesions internally.

Twenty-one birds died of trichomoniasis. At autopsy, the most striking visceral changes were noted in the liver. Only one dying pigeon was seen to have a perfectly clean liver, and cultures from it were negative for *Trichomonas*. Another bird, killed early for class purposes, likewise had no gross liver pathology, though *T. gallinae* grew out in culture. In all the others there was generally very serious hepatic change. The lesions were solid, caseous affairs, as were those of the oral cavity, and ranged from a few large areas which extended deep into the organ, to livers in which the caseation actually represented 90 per cent of the bulk. In some infections the areas were small but extremely numerous. In others both large and small ones were intermingled. The extent to which both lobes of the liver were involved was quite marked.

Cultures from these livers, except in one case, consistently gave bacteria-free growths of *T. gallinae*. Cultivations were made both aerobically and anaerobically. The one exception was the case of a bird which died during the night. Here *E. coli communis* was recovered as well as the *Trichomonas*.

The other viscera appeared to be involved mainly as contact extensions from the hepatic lesions. In two cases large necrotic areas were found on the outer wall of the small intestine where it had been in proximity with a large caseation of the liver surface. In one of these was found the only case of a lesion in the pancreas. It appeared to have developed from direct contact with the diseased gut.

The lungs showed no evidence of infection except in one case. Here the small intestine also bore numerous patches, and the liver was extremely caseous. These lungs were quite extensively attacked.

In four instances the heart wall itself showed caseations. These varied in number, and occurred only on the ventricles. Again, they appeared as direct extensions from adjacent liver lesions.

The blood of four of the most seriously affected birds was examined. Uncontaminated blood was secured by searing the ventricular wall and then jabbing it with a scalpel. From one squab's blood was obtained a pure culture of *T. gallinae*

The body cavity itself usually contained considerable serous exudate, which always yielded *Trichomonas*. Adhesions between the viscera and the peritoneum were common and at times quite extensive. These frequently involved the posterior air sacs. Adhesions involving the pericardium were noted in those cases where the heart wall was affected.

No gross lesions were noted in the spleen, kidney, or on the surface of the large intestine.

At death, the birds were generally severely wasted and quite weak. Occasionally one would appear to be in good flesh, but usually they were "skin and bones." They stood huddled, with feathers ruffled, and when forced to walk would frequently topple over. A noticeable feature of these dying birds was the amount of fluid in their crops. Almost invariably there was an excessive accumulation of greenish liquid, which ran from the mouth on the death of the bird. This always contained large numbers of *Trichomonas*.

In the twenty-one birds dying of trichomoniasis the average bird succumbed on the tenth day following the introduction of the parasite. This is approximately five days after the appearance of the first lesion, which was slightly earlier in these dying birds (average, 5.1 days) than in the total number to which *T. gallinae* was given (average, 7.4 days).

DISCUSSION

Before discussing the lesions found here, especially those of the upper alimentary tract, it is first necessary to mention briefly the anatomical situation in the pigeon's oral cavity. Unlike the condition in man, in which the true oral and pharyngeal areas are clearly differentiated, the pigeon has an oral cavity in which some true mouth and pharyngeal structures are in the same region. For example, the internal nares are located above the folds of the hard palate, and the glottis is very far forward in what is obviously the floor of the mouth. The flaps of the soft palate are situated at the very back of the oral cavity, in an area corresponding to the pharynx. Strictly speaking, then, one cannot refer precisely to "mouth" and "pharynx" in the pigeon's oral cavity and mean distinct areas. For the sake of simplicity the region of the oral cavity anterior to the soft palate has been considered in this paper as the mouth, and the portion to the rear, including the soft palate and just above the oesophagus, has been referred to as the pharynx.

When one examines the published statements concerning the favorite sites of attack and lesion production in *T. gallinae* infections, it becomes apparent that some reanalysis is in order. Benbrook (1945), in his list of parasites of domesticated animals, did not report *T. gallinae* as being present in the respiratory system (lungs), mouth and pharynx, or peritoneum and peritoneal cavity. Hagan (1943) stated that the "most conspicuous lesions are in the liver." Chandler (1944) wrote that "*T. gallinae* of pigeons and many other birds primarily attacks the throat, esophagus, and crop, but may attack many other organs," including the "ducts in the liver and pancreas." Bushnell (1942) believed that whereas lesions may be in the liver, lung, and other tissues, the crop is the main site of attack. Cauthen (1936) found the preferred site of tissue invasion in a series of pigeons, ring and mourning doves to be "the gut between the crop and the glandular stomach. Other important sites were the oral linings, and the liver. As a rule tissue invasion was confined to one organ per bird."

Many more statements could be noted, including some on birds of prey (Stabler, 1941), chickens (Levine and Brandly, 1940), and turkeys (Volkmar, 1930; Hawn, 1937). It is entirely possible, with the differences in the methods of administration of *T. gallinae,* the great diversity in the types of birds used, the differences in the conditions of the birds themselves, i.e., age, presence or absence of a natural infection with *T. gallinae,* that no generalizations can be made on the pathology of *T. gallinae* trichomoniasis.

It seems, however, that something more nearly approaching the natural course of events may be realized from the present series of experiments because: 1) the bird used (pigeon) is the most logical one on epidemiological grounds; 2) the individuals used had had no previous contact whatever with the parasite to be studied; and 3) the method of administration of the parasite as nearly approached the natural process as was possible under the experimental conditions.

Under the above provisions the following general conclusions may be drawn: 1) the earliest visible lesions appear in the mouth cavity, and these are commonest on the soft palate; 2) this cavity is the site of the severest pathology of the alimentary canal, with the pharyngeal region the hardest hit; 3) though less involved than the mouth cavity, in the order of oesophagus, crop, and proventriculus, these regions were occasionally very heavily attacked; 4) the lesions of the mouth not infrequently extended deep into the tissues of the head, involving the eyes and floor of the skull, as well as the soft parts of the head and neck; 5) the alimentary canal from the stomach back was not invaded by *T. gallinae;* 6) in the body cavity, which appeared to be invaded some time after the lesions were well advanced in the mouth and pharynx, the liver was by far the most severely attacked, sometimes being almost completely transformed into caseation; 7) other visceral organs seemed to be involved via direct contact with large superficial hepatic caseations, and variously included lungs, small intestine, pancreas, and the heart itself; 8) the peritoneum was quite generally involved, as evidenced by frequent and extensive adhesions; and 9) the two sites in the whole body which were the most heavily and frequently involved were the pharynx and the liver.

SUMMARY

1. Pigeon squabs (*Columba livia*) averaging seven weeks of age from a loft free of *Trichomonas gallinae* were given oral implantations of a strain of this pathogen (with and without bacteria) isolated from the pharyngeal lesions of a peregrine falcon (*Falco peregrinus anatum*) which had died of the infection.

2. Neither the bacteria isolated from the falcon's mouth (*Escherichia coli communis* and *Micrococcus candicans*) nor those grown from the original *Trichomonas* cultures made from the same source (the two above plus *Bacillus subtilis*) produced any detectable disturbance in any of the twelve birds into which they were put.

3. These same bacteria, when put variously into the mouths of thirteen squabs along with *T. gallinae,* appeared to have no effect whatever on the extent or severity of the lesions produced by the *Trichomonas.*

4. Pure-culture *T. gallinae* of the same strain was placed in the mouths of seventeen squabs. Fifteen died of trichomoniasis; two were killed for culture and demonstration purposes. None survived.

5. Lesions, which appeared in an average of 7.4 days, were first noted as small yellow areas, generally in the mid-line between the soft palatal flaps in the pharyngeal region of the mouth.

6. The mouth cavity was the site of the severest attack of all the pregastric digestive tract, with the pharyngeal portion generally showing the greatest involvement. The postgastric intestine was not found to be internally invaded.

7. At autopsy, the birds dying of trichomoniasis (death occurred on an average of 10 days after the implantation of *T. gallinae*) showed the greatest pathological changes in the liver. At times this organ was almost entirely transformed into caseation.

8. Surfaces of the small intestine, pancreas, lungs, and heart occasionally bore areas of necrosis, apparently as contact extensions of superficial liver lesions.

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PINWORM (*E. VERMICULARIS*) AND OTHER INTESTINAL PARASITES IN COSTA RICAN CHILDREN

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A study of *E. vermicularis* infection in Costa Rica was carried out because no previous report of the incidence of this common helminth was available from that country. The incidence of *E. vermicularis* was compared with that of other intestinal parasites.

Previous observers in Latin America, using efficient methods, reported high incidences of enterobiasis. Corvalho (1928) in Rio de Janeiro, Brazil, examining scrapings from beneath fingernails, found *E. vermicularis* infections in 29 or 22.3% of 130 school children. Cristovao (1941) in São Paulo, Brazil, using cellophane applicators, found *E. vermicularis* infections in 186 or 60% of 309 children, outpatients from 1 to 14 years of age. Cristovao also found high incidences of other intestinal parasites in the same subjects. Osorio and Mazzotti (1940) in Mexico City, using cellophane applicators, found *E. vermicularis* infections in 352 or 51% of 684 day and boarding school children, 6 to 20 years of age. Bacigalupo (1941) in Buenos Aires, Argentina, using cotton applicators, found *E. vermicularis* infections in 164 or 42% of 389 kindergarten children, 3 to 7 years of age. Mazzotti and Quintanar (1943) in Mexico City, using Scotch tape applicators, found *E. vermicularis* infections in 747 or 48% of 1,551 children, 2 to 13 years of age, in homes, schools, and clinics. A low incidence, 3.7%, was found in Mexico City by Mazzotti, Quintanar, and Prida (1943) in 1,000 subjects, 500 of whom were infants 1–36 months of age and 500 of whom were adults, 60 to 65 years of age.

SUBJECTS

The subjects were patients in the children's wards of San Juan de Dios Hospital,[1] which receives patients from all parts of Costa Rica. Nearly all were white.

One hundred and seventeen patients were examined; a total of 92 were included in the study. Nineteen were excluded because they were found to have been treated with anthelmintics within two weeks before the first examination and 6 were excluded because they had no stool examinations. When anthelmintics were administered during a series of examinations, only examinations made before administration of the anthelmintics were considered. Male patients numbered 64, and female 28. Their ages ranged from 1 month to 14 years. Five were one year or less, 41 were 13 months to 5 years and 46 were from 5 to 14 years. More than half the patients, or 51, lived outside of San Jose, the principal city, while 23 lived in San

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Jose, and for 18, the home address was not ascertained. None of the patients were admitted for enterobiasis. Helminthiasis, the most frequent diagnosis, was made in 39 of the 75 cases for whom the principal diagnoses were recorded by the attending physician. Other parasitic diseases were malaria in 9 cases and amebiasis in 8.

METHODS

The cellophane applicator described at the National Institute of Health was used in this study (Hall, 1937). Specimens were taken from the perineal and perianal regions at 5:30 to 9 AM before the patients were washed. From 1 to 8 examinations were made of each patient on as many different days. Thirty-eight were examined once, 30 were examined from 2 to 6 times, and 24 were examined 7 or more times. The total number of specimens was 319. Stool examinations were performed for ova and cysts routinely without concentration methods.

RESULTS OF EXAMINATIONS

Enterobius vermicularis ova were found in 4 cases, or 4.3%, by means of cellophane applicators. The cases carrying *E. vermicularis* ova were not significantly different from the group, except in age. Two were boys of 4 and 11 years, and two were girls 8 years of age. All had 3 or 4 other parasites. All came from different

TABLE 1.—*Number of cases with parasitic ova, larvae, cysts, or trophozoites*

Parasites	Total		Kinds of specimens showing parasites		
	Number	Per cent	Cellophane swab only	Cellophane swab and stool	Stool only
T. trichiura	67	72.8	3	30	34
A. lumbricoides	50	54.3	7	20	23
Hookworm	40	43.5	0	14	26
E. histolytica	14	15.2	0	0	14
Flagellates and ciliates	12	13.0	0	0	12
S. stercoralis	8	8.7	0	2	6
Taenia species	4	4.3	2	1	1
E. vermicularis	4	4.3	4	0	0

places. Only one was living in San Jose, and this was a Nicaraguan, who had been living in the city less than one year. The *E. vermicularis* ova were not numerous in any case, ranging from 3 to 20 in one cellophane preparation. Repeated examinations were made in three cases, two being examined twice, and one being examined six times. No further positive specimens were obtained from any of the three, but this may have been due to the administration of carbon tetrachlorid after the first cellophane swab and stool examination in the patient examined six times. None of the patients had symptoms referable to *E. vermicularis* infection.

A high incidence of other helminth ova and protozoan cysts was found. From 1 to 4 parasites were present in each of 74 cases, or 80%. Nearly all were found by routine stool examinations, although the cellophane swabs disclosed evidence of the presence of additional parasites not observed in the stools in 13 cases, and were the only evidence of infection in 3. The types of ova and cysts that were recognized, and their frequency, are given in Table 1 in the order of frequency. *Trichuris trichiura, Ascaris lumbricoides,* and hookworm predominated. Many of the infections were heavy.

The cellophane swabs were most efficient for detecting *E. vermicularis* infections which in this series were found only by this means (Table 1). They were also of some value for other ova, but not for helminth larvae, protozoan cysts, or trophozoites. In the case of *Taenia* ova the cellophane swab was more useful than the routine stool examination. Two of the 4 cases with *Taenia* ova were recognized only by means of cellophane swabs, one only by stool examination, and one by examination of both kinds of specimens.

DISCUSSION

A high incidence of *E. vermicularis* infection has been found in children of school age in Latin America and in all parts of the world where examinations have been carried out. In reporting a low incidence in the first study in Costa Rica in a small group of hospitalized children, one cannot assume that the results are representative of the country. Studies of families in which one member is found infected, or of child care institutions, may show higher incidences.

The inclusion of infants tends to make the total incidence low. No infections were found in the 38 patients less than four years of age. When only the subjects four years of age and older are included, the incidence is also low, namely 4 out of 54 children, or 7.4%.

Hospitalization may have a tendency to obscure or cure infections with *E. vermicularis*. In two previous studies on hospitalized patients, a relatively low incidence was also found, namely: Jones (1942), incidence 14%, and Headlee (1943), incidence 16.3%. In the former study the question was raised as to whether hospital procedures such as enemas, rectal temperatures, or washing and bathing were responsible for the relatively low incidence, or prevented the detection of some infections. No evidence for such influence was obtained in the present study.

The types of homes in which the Costa Rican patients lived differed in several respects from those in which high rates of infection have been found elsewhere. The majority of the patients lived in rural areas and none lived in large cities. Although there was some crowding in the individual homes, the climate did not make it necessary for the children to spend a large proportion of the time indoors at any season. Dry heat of 27 to 29° C with 38–50% humidity kills *E. vermicularis* eggs experimentally in 40 hours (Jones and Jacobs, 1941). These conditions may occur in the dry season in many parts of Costa Rica.

The children had ample room for play and therefore may have had less personal contact, and most of such contact occurred out of doors in a dry, sunny climate. Clothes were less elaborate than those required in more rigorous climates and thus did not furnish as good opportunities for the concentration of ova. In these respects, the living conditions of the Costa Ricans differ from those in cities and institutions which have been the subject of most of the previous studies and may be analogous to those of colored children in the Southern United States among whom a comparatively low incidence has also been found (Cram, 1940, and Jones, 1942).

The presence of a low incidence of *E. vermicularis* infection among children with a high incidence of other intestinal parasitic infections serves to emphasize the difference in the mode of transmission. It appears that the conditions for the spread or development of *E. vermicularis* infection may be absent even though conditions for the spread or development of other helminths are favorable.

SUMMARY

An unusually low incidence of *E. vermicularis* infection of 4.3% was found in 92 hospitalized children in San Jose, Costa Rica, among whom the incidence of other intestinal parasitic infections was high.

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HELMINTHS OF ARMADILLOS, *DASYPUS NOVEMCINCTUS*, IN EASTERN TEXAS

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Through the kindness of Mr. Rollin H. Baker, Field Biologist for the Texas State Game, Fish, and Oyster Commission, a series of eight armadillos from eastern Texas was provided for helminthological examination.

Although the armadillo is almost exclusively insectivorous, and remains of insects of many different kinds were found in the contents of the stomach and intestine, it is remarkable that not a single adult helminth was found that is believed to require an arthropod as an intermediate host. Actually, only two adult helminths were encountered; (1) a fluke, *Brachylaemus virginianus,* previously recorded in nature only from opossums, which reaches its infective stage in snails (*Polygyra* and others), and (2) an oxyurid, *Aspidodera fasciata,* which presumably requires no intermediate host. This dearth of adult helminths requiring arthropod intermediate hosts is in striking contrast to the condition in other mammals living in the same general environment, e.g., opossums and raccoons.

According to the host records of the Zoological Division of the U. S. Bureau of Animal Industry, this situation is general for the Dasypodidae. The only adult worms presumably requiring ingested arthropod intermediate hosts reported from armadillos are the anoplocephalid tapeworm, *Oochoristica surinamensis,* and the acanthocephalan, *Travassosia carinii.* Stiles and Hassall (1894) report an *"Echinorhynchus sp."* from *Dasypus novemcinctus,* collected by Hassall in Texas, and in the collection of the Bureau of Animal Industry. Dr. E. W. Price, in correspondence, reported to the writer that he could find no record of this collection in the catalogue of the Helminthological Society, so it is now uncertain whether this record refers to larval forms of *Oncicola,* or to an adult worm in the intestine.

On the other hand, three worms which presumably require arthropod intermediate hosts—the spiruroid nematodes, *Physocephalus* and *Ascarops,* and an acanthocephalan, *Oncicola canis*—were found as encysted larvae in the mesenteries or cecal walls. The spiruroids are normally parasitic in pigs and reach the infective stage in dung beetles, and *Oncicola* is normally parasitic in dogs and coyotes, and presumably reaches the infective stage in some arthropod. In this connection it is interesting to note that Cameron (1939) reported finding larval specimens of another spiruroid, *Physaloptera* sp., in an armadillo in Trinidad. Still another parasite, *Porocephalus crotali,* has been reported as encysted larvae in armadillos in Brazil.

Armadillos in which large numbers of the encysted larvae of spiruroids were found were captured in areas where pigs were allowed to roam. Since it is unlikely that the armadillos are often killed and eaten by pigs, and since many of the encysted worms were dead and often undergoing calcification, it must be concluded that in hog pastures armadillos have a protective effect by picking up infected arthropod hosts and thus sidetracking the larvae that might otherwise eventually be eaten by the pigs.

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The helminths found in the eight armadillos examined were as follows:

Brachylaemus virginianus ..... in 3 specimens
Oncicola canis, encysted larvae ..... in 5 specimens
Hamanniella sp. (*tortuosa?*), immature intestinal forms ..... in 1 specimen
Aspidodera fasciata ..... in 6 specimens
Physocephalus sp., encysted larvae ..... in 3 specimens
Ascarops sp., encysted larvae ..... in 2 specimens

Brachylaemus virginianus.—Flukes which are identical in every respect with the *Brachylaemus* described by the writer (1932) from Texas opossums, *Didelphys virginiana,* under the name *Harmostomum opisthotrias,* were found in three of the eight armadillos examined, but not in as large numbers as they usually occur in opossums.

A *Brachylaemus* was first described from North American opossums by Dickerson (1930) in Virginia, under the name *Harmostomum opisthotrias* var. *virginianum.* Sinitsin (1931) found it abundantly near Washington, D. C., and referred to it as *H. migrans,* a species described from European shrews. Krull (1934, 1935) gave it the rank of a distinct species, *Brachylaemus virginianus.* Meanwhile the writer (1932) found many of the measurements of specimens found in opossums in Texas to be intermediate between those of *B. opisthotrias* of Brazil and those of Dickerson's Virginia specimens, and thought it doubtful that the latter represented even a distinct variety, the size differences possibly being due to different intermediate hosts. Subsequently, however, Dollfus (1935) described and figured two specimens of *B. opisthotrias* from Brazil which provide better measurements than were previously available. The much larger size and much larger suckers of this fluke, with the acetabulum slightly larger than the oral sucker, leaves little doubt that the North American specimens are not co-specific with the Brazilian ones. Examination and measurement by the writer of additional specimens from Texas opossums and armadillos, of additional specimens from Virginia kindly supplied by Dr. B. D. Reynolds, as well as measurements of specimens from Maryland opossums given by Krull (1935), show clearly that all the North American specimens from naturally infected opossums and armadillos represent a single species. In the writer's original description of the forms from Texas opossums it was stated that no spines were present on the anterior end of the body, but spines can be seen clearly in fresh living specimens and in specimens placed in fixative while still alive.

Meanwhile, however, Krull (1933) was able to infect opossums with *B. spinosulus* (Hofmann, 1899), a species occurring in European hedgehogs. Krull (1934) was also able to infect young dogs, cats, rats, and chickens with *B. virginianus,* and observed a marked difference in size between flukes of the same age in puppies and kittens. It appears, therefore, that *Brachylaemus* is very indiscriminate with respect to its definitive host, a fact that was pointed out by Sinitsin in 1931. Krull (1935) gave comparative measurements of various organs of *B. spinosulus* from experimentally infected opossums, and of *B. virginianus,* and concluded that there were sufficient differences to warrant retaining them as distinct species until adequate descriptions and life history work make possible a final judgment.

All the measurements given by Krull for *B. spinosulus,* however, are within the range of measurements obtained by the writer from specimens of *B. virginianus* from naturally infected opossums in both Virginia and Texas, and from Texas armadillos.

The writer, therefore, thinks it very probable that the *Brachylaemus* occurring in opossums and armadillos in the United States will prove to be identical with *B. spinosulus* of European hedgehogs. There is also some reason to believe that some of the forms described from European shrews, and perhaps even from birds, represent the same species. The extent to which specimens of a single species of this genus vary in different hosts, and as a result of the state of contraction, and whether dead or alive when fixed, makes the whole question of speciation within the group very questionable. For the present the writer agrees with Krull that the name *B. virginianus* should tentatively be retained for the species found in North American opossums, and here reported from armadillos, although it seems probable that it will eventually have to fall in synonymy with one or more of the earlier-described European species.

Oncicola canis.—The occurrence of encysted larval forms of this parasite in five of the eight opossums was not surprising, since it had previously been reported from this host in Texas by several authors. A high percentage of the *Oncicola* larvae, however, were dead, and frequently calcified. Dead larvae were found in every armadillo that contained specimens of this worm, and in two instances *all* the larvae were dead. It seems possible, therefore, that in this case, as in that of the spiruroids mentioned above, the armadillo may serve as a means of destruction of infected intermediate hosts, with ultimate death of the parasites, more frequently than it serves as a transport host through the mediation of which dogs and coyotes become infected. Van Cleave (1920) in commenting on the exceptional occurrence of a specimen of *Oncicola* in a dog at Lincoln, Nebr., assumed that the armadillo played an essential rôle in the life cycle, although he recognized the strong probability of a prior arthropod host, and Price (1926) accepted this hypothesis, and thought it explained the limitation of distribution of this parasite in the United States to Texas, beyond which the armadillo does not range. Of course, this coincidence of distribution, though suggestive, does not necessarily point to the armadillo as a necessary link in the life cycle of *Oncicola*. Other species of *Oncicola* occur in FELIDAE in Africa and Malacca, where there are no armadillos. Price's (1929) report of encysted larvae in the esophagus of turkey poults throws further doubt on the essential rôle of armadillos.

Hamanniella sp. (probably *tortuosa*).—In one armadillo three young intestinal forms of an acanthocephalan were found. The identity of these worms was at first very doubtful, but comparison with worms of similar size from an opossum, in which a complete series of specimens of *Hamanniella tortuosa* of progressive size and age were found, leaves no room for doubt that these worms belong to the same genus, and probably represent the same species, having been able at least to initiate their growth in the armadillo's intestine. *Hamanniella tortuosa* adults have not been reported from armadillos, although a related worm, *Travassosia carinii,* is parasitic in armadillos in South America.

The three specimens found are all males, and appear to be of approximately the same age; presumably they were acquired from a single intermediate host. They measure 7 to 9 mm in length and 350 to 440 microns in width. The proboscis and proboscis sac are typical of *Hamanniella tortuosa*. The lemnisci are uniformly about 5 mm long, regardless of the length of the body. The testes are very small, the anterior one 2.1 to 2.5 mm, and the posterior one 1.33 to 1.75 mm, from the posterior

end. In the smallest specimen, one lemniscus reaches the anterior testis. The cement glands have not yet differentiated but their position is indicated by a thickening of the tissue along the vas deferens between the testes and the ejaculatory duct. In the least developed specimen only eight large nuclei are visible, probably representing the eight cement glands that will eventually develop, but some of these are undergoing division. In the other specimens the nuclei in the cement gland complex are more numerous and still undergoing division. At the anterior end of the so-called ejaculatory duct and just posterior to the cement gland complex is a bladder-like structure with two bladder-like lobes, giving the appearance of three lobes. These were at first thought to be the developing cement glands but Van Cleave, after examining them, correctly stated (in correspondence) that they were not. Their true nature, and the development of the entire male reproductive system, of *H. tortuosa,* as observed in the series of specimens from the opossum, will be discussed in another paper.

Aspidodera fasciata.—This is the only parasite found in this series of armadillos that can be considered specific for this host. This worm, found in considerable numbers in the cecum and upper part of the large intestine of all but one of the armadillos examined, has not hitherto been found in North America, but has been reported from *Dasypus novemcinctus, Tolypeutes tricinctus* and *T. conurus* in Brazil and Bolivia. The Texas specimens correspond very closely with the description and figures of this worm given by Proença (1937) but differ in a few minor details, though not sufficiently to warrant the erection of a new species. The arrangement of the cephalic cordons corresponds exactly to the figure given by Proença. Most of the measurements agree but the specimens of both sexes average smaller in size, and in the females the tail is from 350 to 485 microns in length as compared with 490 to 530 microns as given by Proença. In the males the tail is 350 to 484 microns long, the spicules 290 to 325 microns long, and the gubernaculum 105 to 125 microns long; the corresponding measurements for the South American forms are: tail 490 to 530 microns, spicules 320 to 360 microns, gubernaculum 140 to 159 microns. The arrangement and number of caudal papillae in the male corresponds as far as they could be observed, but some of the small laterally placed preanal papillae figured and described by Proença were not seen.

Physaloptera sp. and *Ascarops* sp.—As noted above, encysted larvae of these pig parasites, frequently dead and calcified, were found in the mesenteries and cecal walls of several armadillos obtained from pig pastures. The significance of this finding has already been referred to.

SUMMARY

In a series of eight armadillos examined, only one worm was found, *Aspidodera fasciata,* for which this mammal can be considered the normal definitive host. The only other adult worm was the fluke, *Brachylaemus virginianus,* normally parasitic in oppossums. Immature intestinal specimens of a *Hamanniella,* found in one armadillo, are probably misplaced specimens of *H. tortuosa,* also normally parasitic in opossums. Encysted larvae of two spiruroids of pigs, *Physocephalus* and *Ascarops,* and encysted larvae of *Oncicola canis,* were found in a number of the animals. In all these cases, since the majority of the larvae were dead and many calcified, it is believed that the armadillo serves as a means of destruction of these parasites rather than as a transport host.

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OBSERVATIONS ON THE ANATOMY OF MESOCESTOIDES

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The genus *Mesocestoides,* the only genus in the family Mesocestoididae, is peculiar among cyclophyllidean tapeworms in two respects: (1) the position of the genital atrium on the median line of the ventral surface, and (2) the possession of two entirely separate yolk glands. Another peculiar feature is the concentration, in gravid segments, of all the eggs in an egg ball enclosed by a thick fibrous capsule in what all previous observers and writers have considered the posterior portion of the uterus.

Some authors have suspected other peculiar features, though these have not been generally accepted. For example, Hamann (1885) and Muehling (1898) considered the zone of darkly staining cells from which the egg capsule forms to represent the Mehlis' glands, and Mueller (1928) stated that he believed this to be correct. Cameron (1925), however, in two new species of *Mesocestoides* described by him (*M. mesorchis* and *M. caestus*), observed a small shell gland surrounding the oviduct close to the point where it joins the uterus. In a later paper Mueller (1930) reported that in *M. latus* "the ovaries are in the form of a bilobed mass, confluent near their middle or posterior level, with the shell gland in their anterior angle."

Another point of dispute has been the presence or absence of vasa efferentia leading from the proximinal end of the coiled vas deferens to the testes. Zschokke (1889) represented a highly developed system of such vessels, whereas Mueller (1928) says "Certainly such does not exist, Zschokke probably having been misled by the coiling proximal end of the vas deferens. In my specimens the testes at the height of activity show a disintegration of their walls and it seems probable that at this time the sperms migrate through the loose parenchyma to the vas deferens." In his description of *M. latus* in the same paper he stated "When the sperm cells mature the testicular walls are largely resorbed, and clusters of spermatozoa are seen apparently loose in the parenchyma."

The writer was fortunate in obtaining a fresh specimen of *M. latus* from an opossum which, when stained and mounted, showed details of structure in a manner that is very unusual, and made possible observations which I have not been able to make on other specimens of this or other species of the genus. These observations have made it possible to elucidate the disputed points mentioned above, and to show that in one point at least all of the previous descriptions of the anatomy of this worm have been erroneous.

In *Mesocestoides latus,* more than in other species of the genus that have been studied by the writer, the glands and ducts of the reproductive system tend to persist in gravid segments in a sort of "mummified" form. This characteristic is especially conspicuous in the specimen on which these observations are based. The ovaries and vitelline glands, vagina, oviduct, vitelline duct, oötype, testes, vas deferens and cirrus pouch are all excellently preserved in these gravid segments, and are rendered visible to an unusual degree by the presence in them of minute black

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granules. The ovaries, vitelline glands and testes are empty shell-like structures enclosing these granules.

In addition to these structures there is also visible a uterine duct leading from the oviduct to the uterus, connecting with the latter near its termination, anterior to what has previously been called the uterine capsule or egg ball. It becomes perfectly evident that the latter structure is not the posterior portion of the uterus, as all previous writers have said, but is a true parauterine organ. Fuhrmann (1928–1933) after describing parauterine organs in other tapeworms stated the universally-held opinion concerning this organ in the following words: "Ganz anders liegen die Verhältnisse bei *Mesocestoides* Vaillant. Der schlauchförmige Uterus, der in der Medianlinie mehr oder weniger gewunden verläuft, zeigt am Hinterende (Figur 414) eine radiäre fibröse Hülle, die später, wenn sich alle Eier vom Vorderteil des Uterus im Hinterende angesammelt haben, zur meist sphärischen und geschlossenen Uterin-kapsel wird, die funktionell identisch ist den Paruterin-Kapseln der obigen Formen."

When the fact that this structure was a true parauterine organ became apparent from a study of the "mummified" remains of the organs in the gravid proglottids, a careful study of the sexually-functional early segments showed that the development of this structure, except for its posterior instead of anterior or lateral position, agrees with Fuhrmann's description of its development in other genera. It forms independently of the uterus, between it and the ovary in the median line of the proglottid, as a solid mass of deeply-staining cells. It soon acquires a cavity and then establishes a broad connection with the uterus at a point posterior to the entrance of the uterine duct (Fig. 2, p.ut.d.). Subsequently the walls condense and become fibrous, leaving a twisted passageway to the parauterine duct, as previously described by other writers. This is shown in Fig. 1.

The posterior end of the uterus, posterior to the entrance of the uterine duct, remains as a thin-walled tube even after the parauterine capsule is fully developed and contains nearly all the eggs. The rest of the uterus, however, undergoes a change, the original thin wall becoming surrounded by a rather thick coat of what appear to be glandular cells, which stain very deeply. This development is more conspicuous in *M. latus* than in other species but has also been observed in *M. variabilis* and *M. kirbyi,* though not in *M. manteri.*

In young, sexually-active segments the uterine duct is a thin-walled, tortuous tube of large bore which lies dorsal to the developing parauterine organ, and dorsal even to the vagina (Fig. 2, ut.d.). It is seen to contain ova which are evidently en route from the ovaries to the uterus.

The arrangement of the ducts in the ovarial region has never been carefully or correctly described, being very difficult to see in most specimens. The close approximation of the ovaries, which are often contiguous, further obscures this area. In the gravid segments, however, the details of this area are easy to see, especially in the specimen on which the present description is based. The arrangement of these ducts is shown, only slightly modified from their usual position, in Fig. 3.

The vagina, although a rather broad tube in young segments, becomes a narrow one in the gravid segments. In the ovarial region it branches into two broader tubes, at the head of an arch. One of these tubes, the oviduct, runs posteriorly a short distance, then turns sharply ventrad and then forward, then turns dorsad to an ex-

panded area which represents the oötype, and which in young proglottids is surrounded by the Mehlis' glands (Fig. 2, M.gl.). From the oötype this tube continues dorsad and enters, from the ventral side, the transverse tube which represents the ovarian bridge. In the younger proglottids, such as the one from which Fig. 2 was drawn, this bridge is not clearly seen. The Mehlis' glands and oötype, situated almost directly ventral to the ovarian bridge, are therefore not in the position where they are usually found in cestodes, which is distal to the junction of the vitelline duct with the oviduct. Their position in Fig. 389 of Fuhrmann (1928–1933), representing *M. lineatus,* where the Mehlis' glands are in their usual position beyond the entrance of the vitelline duct, is so different that it is difficult to believe that it can be correct. Such a variation would not be expected in two such closely related species. The position described for them in *M. latus* by Mueller, in the anterior angle of the bilobed uterine mass, is correct as far as it goes, but Mueller does not indicate their true relation to adjoining organs.

The other leg of the arch into which the vagina opens bends posteriorly and then dorsad, and then continues forward in a dorsal position as the uterine duct. It is parallel with the vagina, often lying directly above that tube, and continues forward, with only slight undulations in gravid segments, to its junction with the true uterus. The common vitelline duct (Fig. 3, c.v.d.) empties into this tube a short distance after it turns forward. The common vitelline duct divides after a short course posteriad, one branch running to each vitelline gland.

The "mummified" vas deferens is very conspicuous in gravid segments. It runs forward in a tortuous manner from the cirrus pouch to a level about midway between that organ and the anterior end of the uterus, then turns posteriad to the anterior part of the parauterine organ, where it divides into two nearly right-angle branches. These then branch again repeatedly to form the vasa efferentia, which are extremely fine. A few of them could be traced directly to the dried shells of the testes. It is clear, therefore, that Zschokke was correct with respect to the presence of vasa efferentia, though these are not visible in sexually active proglottids; it is only after they have dried, and are marked by the same minute black granules visible in other ducts, that they can be observed. The sperms that Mueller reported having seen apparently free in the parenchyma may have been liberated from crushed testes in a flattened specimen, or they may have been present in the vasa efferentia which were not visible because of their fineness and thin walls.

SUMMARY

Examination of a specimen of *Mesocestoides latus* in which the reproductive glands and ducts are particularly well shown in gravid segments, showed that the so-called uterine capsule is a true parauterine organ, and that there is a uterine duct connecting the oviduct with the true uterus, which had not previously been observed. The oötype and Mehlis' glands are situated in an unusual position on the oviduct just after it leaves the ovaries, proximal to the entrance of the common vitelline duct. The vas deferens branches near the anterior end of the parauterine organ, and each branch gives rise to branching vasa efferentia which connect directly with the testes.

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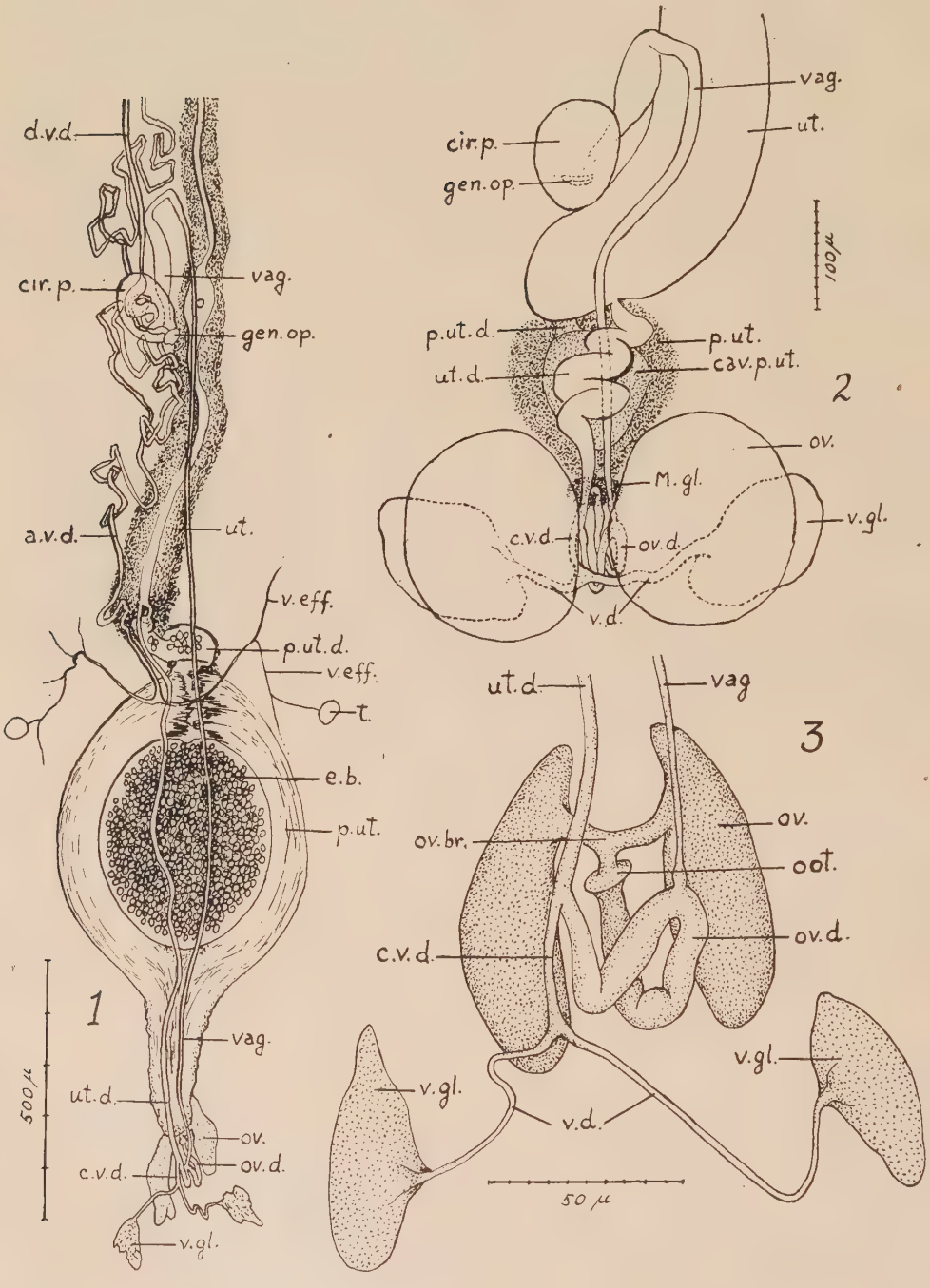
Explanation of Plate

FIG. 1. Reproductive organs of *Mesocestoides latus* as they appear in a gravid proglottid, dorsal view. Note uterine duct, and separate openings of this and parauterine organ into posterior end of true uterus. The coils of the descending vas deferens as they pass dorsally over the cirrus pouch are not shown.

FIG. 2. Reproductive organs of *M. latus* as they appear in a sexually-active young proglottid. The ovarian bridge, which lies dorsal to the Mehlis' glands, was not visible and is not shown.

FIG. 3. Arrangement of reproductive glands and ducts in posterior region of a gravid proglottid. All the structures are present, dried and empty, except the Mehlis' glands surrounding the oötype, which have disappeared.

Abbrev.: *a.v.d.*, ascending vas deferens; *cav.p.ut.*, cavity of parauterine organ; *cir.p.*, cirrus pouch; *c.v.d.*, common vitelline duct; *d.v.d.*, descending vas deferens; *e.b.*, egg ball; *gen.op.*, genital opening; *M.gl.*, Mehlis' glands; *oot.*, oötype; *ov.*, ovary; *ov.br.*, ovarian bridge; *ov.d.*, oviduct; *p.ut.*, parauterine organ; *p.ut.d.*, parauterine duct; *t.*, testis; *ut.*, uterus; *ut.d.*, uterine duct; *vag.*, vagina; *v.eff.*, vasa efferentia; *v.d.*, vitelline duct; *v.gl.*, vitelline gland.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THE USE OF MIXED COLONIES OF TERMITES IN THE STUDY OF HOST–SYMBIONT RELATIONS

VICTOR H. DROPKIN, PH.D.[1]

The termite-protozoa complex is interesting to the student of evolution as an illustration of the evolution of a sexually reproducing group, the termites, paralleled by the asexually reproducing protozoa. These protozoa were present in the intestines of their insect hosts well before the Cretaceous, for they occur in the wood roach, *Cryptocercus punctulatus* Scudder as well as in the more primitive families of termites. In general, each species of host termite has its own distinctive faunule of protozoa. The present study analyses the behavior of the termite host as an isolating mechanism for the protozoa.

A termite colony in nature is composed of a single species of termite. It often happens that the galleries of two separate colonies interdigitate in the same log, but they never run into each other. In all the observations in nature, no colony has been found containing more than one species of termite. The typical reaction when members of different species of termites are brought together in nature and in the laboratory is a violent, mutual attack. The normal behavior patterns of the insect hosts were modified in these experiments to permit the formation of laboratory colonies consisting of two or more species of termites.

Laboratory colonies are kept in thin plywood between panes of glass to permit observation of the termites at all times. The plywood is drilled at the center to provide space for the insects in starting the colony. After several days the galleries extend out from the center through the plywood. A sheet of paper towelling between the wood and one pane of glass serves to hold sufficient moisture. The glass is bound with tape, and the whole colony fits into a metal cigarette tin measuring $4.5'' \times 5.75'' \times 0.5''$. This method of keeping termite colonies is similar to the one suggested by Adamson (1941).

All the colonies were kept in the same type of plywood for several months before mixing. On the day of an experiment, the stock colonies (each consisting of a single species) are thoroughly chilled in the refrigerator to immobilize the insects. The desired number of chilled termites of the foreign species are introduced into the colony. As the temperature rises the insects become active. If fighting is noted at this time, the mixed colony is returned to the refrigerator. Several successive chillings are usually sufficient to permit the establishment of a mixed colony. Once the additions to the colony have been accepted no distinction can be noted in the behavior of the termites towards members of their own species as compared with members of the introduced species. Species recognition among termites is probably an odor-taste phenomenon.

The following combinations of two species in the same colony were achieved:

Kalotermes jouteli Banks with: *K. schwarzi* Banks
K. snyderi Light

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[1] Acknowledgment is made to Dr. A. E. Emerson, University of Chicago, who acted in an advisory capacity throughout the work, and to Dr. E. M. Miller, University of Miami, Fla., for aid in obtaining living termites.

K. tabogae Snyder
Neotermes castaneus Burmeister
Zootermopsis sp.

Kalotermes schwarzi with: *K. snyderi*
K. tabogae
Neotermes castaneus

Zootermopsis sp. lived with *Prorhinotermes simplex* (Hagen). One colony was successful with *K. jouteli, K. schwarzi,* and *Neotermes castaneus* all in the same galleries.

Those termites which have flagellate symbionts lose their infections at each molt. After each molt the insect reacquires protozoa by feeding on the fluid excretions from the non-molting members of the colony. Hence the method of mixed colonies provides a test for the survival of protozoa naturally foreign to the host. Light and Sanford (1928) showed that protozoa from *Kalotermes hubbardi* Banks could live in *Zootermopsis* sp. for 100 days. There was some evidence of multiplication in the foreign host. Cleveland et al (1934) state that transfaunation was possible between *Zootermopsis* sp. and the roach *Cryptocercus,* and that the protozoa survived at least for a limited time. On the other hand in the experiments of Dropkin (1941) the protozoa of *Reticulitermes flavipes* (Kollar), *Kalotermes jouteli,* and *K. schwarzi* failed to survive for more than 40 days in *Zootermopsis* sp.

The species of termites used were chosen for these experiments because their protozoa are easily distinguished. The larger protozoa of *K. jouteli* are: *Macrotrichomonas restis* Kirby, *Metadevescovina nudula* Kirby, *M. turbula* Kirby, and *Staurojoenina* sp. *K. schwarzi* has an entirely different faunule: *Metacoronympha senta* Kirby, *Coronympha octonaria* Kirby, and *Trichonympha chattoni* Duboscq and Grassé.

Tables 1 and 2 present the data for the colonies containing *K. schwarzi* and *K. jouteli.* Fifty-five per cent of *K. schwarzi* acquired at least some foreign protozoa, as compared with only 23% of *K. jouteli.* In addition, 70 termites have been examined from mixed colonies of *Neotermes castaneus* and *K. jouteli* varying in age from 10 to 304 days. Twenty-two of 36 *K. jouteli* acquired at least some *Neotermes* protozoa, whereas only 5 of 34 *Neotermes* showed other protozoa than their own. Five of the *K. jouteli* contained entirely foreign protozoa.

Both series of experiments show an unequal movement of protozoa. Early in the work it appeared that *K. jouteli* did not acquire foreign protozoa readily, whereas *K. schwarzi* often had a mixed population of foreign and indigenous forms, and sometimes contained entirely foreign protozoa. There were 18 colonies which had equal numbers of individuals of each species of termite. These showed transfer from *K. jouteli* to *K. schwarzi* in 50% of the cases while the reciprocal cross appeared in only 25% of the individuals examined. Furthermore, if we delete those individuals which had recently molted, the non-reciprocal crossing becomes more apparent. Thus 48% of the *K. schwarzi* examined in this series of balanced mixtures maintained their infections with foreign protozoa as contrasted with only 9% of the *K. jouteli.*

In order to establish the fact that *K. jouteli* termites have the opportunity to acquire foreign protozoa when living in mixed colonies, newly molted individuals were marked with asphalt paint on the head and abdomen to make possible the ob-

TABLE 1.—*Number of* K. jouteli *termites in mixed colonies showing transfer of protozoa*

Normal host of protozoa	Age of mixed colony in days													Young forms	Total	%
	1–20	40	60	80	100	120	140	160	180	200	220	240	340	360			
K. jouteli (no transfer)	8	..	..	2	2	5	..	10	..	2	7	13	4	7	1	61	77
K. jouteli, K. schwarzi (some transfer)	10	..	..	..	..	..	..		..	1	..	2	1	..	3	17	22
K. schwarzi (complete transfer) .	1	..	..	..	..	..	..		..	..	..	..	..	..	..	1/79	1

TABLE 2.—*Number of* K. schwarzi *termites in mixed colonies showing transfer of protozoa*

Normal host of protozoa	Age of mixed colony in days													Young forms	Total	%
	1–20	40	60	80	100	120	140	160	180	200	220	240	340	360			
K. schwarzi (no transfer)	7	4	..	..	2	4	3	..	1	1	9	5	5	14	1	56	45
K. schwarzi, K. jouteli (some transfer)	14	8	..	..	2	3	1	2	7	2	3	10	2	1	..	55	44
K. jouteli (complete transfer) .	1	2	..	1	..	3	..	2	..	..	3	2	..	..	..	14/125	11

servation of individuals at known intervals after molting. These observations showed that protozoa do arrive in *K. jouteli* from *K. schwarzi*. The nature of the resistance of *K. jouteli* toward protozoa from *K. schwarzi* is not known. Some idea of the length of life of the foreign protozoa was provided by the marked termites. No foreign protozoa were found in marked *K. jouteli* after the 16th day post-molt. The marked *K. schwarzi* however, were found to contain living protozoa from the foreign host on the 35th day.

Another attempt was made to arrive at the length of life of the protozoa after transfaunation by examining the intestines of *K. schwarzi* termites which had molted in mixed colonies and were then removed from the presence of *K. jouteli*. The last termite was killed on the 131st day after removal from contact with the foreign species. It contained two species of protozoa from *K. jouteli* (*Metadevescovina nudula* and *M. turbula*) and one of its own species (*Trichonympha* sp.).

Finally, the records of three of the colonies are interesting because they suggest that the foreign protozoa may be even more successful than the indigenous forms in a *K. schwarzi* termite. Note that in Table 2, 14 *K. schwarzi* are recorded with entirely foreign protozoa. Colony 144 began with 6 *K. jouteli* and 18 *K. schwarzi*. After 230 days the colony contained 5 *K. jouteli* (including 2 young), and 11 *K. schwarzi*. All the individuals were examined. Two of the *K. jouteli* had their own faunules; two (including one young) had protozoa from both species; and one young lacked protozoa. Of the *K. schwarzi*, two had only the indigenous forms, while 7 contained protozoa from both hosts and two individuals had entirely foreign protozoa. At the end of 230 days of life in a colony containing more than twice as many *K. schwarzi* as *K. jouteli*, the protozoa from the latter had invaded 9 of 11 foreign hosts and had completely displaced the indigenous forms in two individuals. Colony 103 consisted of 8 *K. schwarzi* which had been in the presence of *K. jouteli* for 161 days in the proportion of two *K. schwarzi* to one *K. jouteli*. Seven of the eight *K. schwarzi* showed mixed faunules while one underwent no transfaunation. Finally, Colony 66 started with 19 *K. schwarzi* and 5 *K. jouteli*. After 154 days this colony had 1 *K. jouteli* and 10 *K. schwarzi*. The lone *K. jouteli* had only protozoa normal to its species. Three of the *K. schwarzi* nymphs had faunules consisting solely of foreign protozoa. Fifty-two days later, five *K. schwarzi* were alive, of which three had their own protozoa, while one contained mixed protozoa and one had a completely foreign faunule.

It seems then that some resistance to cross infection has evolved during the evolution of these species of termites. This resistance is not equal in both species and is probably not the isolating factor in nature. Apparently the behavior of the termites, although easily overridden in the laboratory, is a factor that tends to isolate the protozoa of each species of host.

SUMMARY

1. The normal behavior patterns of some primitive termites were modified by the use of refrigeration to permit the establishment of mixed colonies. Nine combinations of two species in the same colony, and one combination of three species lived successfully in laboratory colonies.

2. Life in a mixed colony affords the opportunity for a recently molted termite to acquire protozoa naturally foreign to its species. *Kalotermes jouteli* demonstrated

resistance to infection with protozoa from *K. schwarzi*. But the reciprocal cross succeeded, even in cases where the colony contained few *K. jouteli* and many *K. schwarzi*.

3. The behavior of the termites, rather than the resistance to transfaunation is considered to be the factor which isolates the faunules of these species of termite.

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THE LABORATORY REARING OF THE TROPICAL RAT MITE, *LIPONYSSUS BACOTI* (HIRST)

ROGER W. WILLIAMS[1]

Heretofore, certain phases of filariasis research have been closed to investigators for lack of a suitable laboratory animal which could be experimentally infected easily and quickly with a filariid parasite. Williams and Brown (1945, 1946) have shown that the tropical rat mite, *Liponyssus bacoti* (Hirst, 1913) is an intermediate host of the cotton rat filariid, *Litomosoides carinii* (Travassos, 1919). Through this mite, cotton rats (*Sigmodon hispidus*), which are not infected with the filariid, and the common albino rat can be infected in a relatively short period of time when kept in close proximity with infected animals. When colonies of mites are produced in the laboratory induced filarial infections permit investigators of filariasis to conduct prophylactic and immunological studies as well as studies pertaining to the age resistance of adult worms to various chemotherapeutic agents.

Dr. E. W. Baker, in recent correspondence, states that *L. bacoti* has a wide distribution both in the United States and elsewhere. He reports that records at the United States National Museum indicate that it has been found in 34 of the 48 states, namely, Alabama, Arizona, Arkansas, California, Delaware, Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Washington, West Virginia, Wisconsin, and also from the District of Columbia. Outside of the continental United States it has been taken in Abyssinia, Argentina, Australia, Brazil, Canada, Colombia, Dutch New Guinea, Egypt, England, Formosa, Hawaii, Honduras, Liberia, Mexico, "Orient," Panama, Peru, and Puerto Rico. The reported rodent hosts in the United States are rats, mice, guinea pigs, and squirrels.

Since it is not feasible to collect in nature a sufficient number of *L. bacoti* for transmission experiments, it is necessary to develop colonies by rearing these arthropods in the laboratory in order to have a continual source of supply. The initial group of mites with which to begin colonies may be collected from the rodent nests or after dropping from wild-caught rodents. One would expect to find greater numbers of mites in the nests for, as Bishopp (1923) points out, these mites feed largely when the animals are quiet in their nests and the majority of them detach themselves and remain in the nests. However, sufficient numbers of mites to begin a colony can be obtained from live-caught animals. As soon as possible after catching, the rodents should be placed in individual wire mesh cages on fine wood shavings. Twenty-four hours later the shavings may be examined for mites by scattering them on white paper and probing or "combing" them with an applicator or any long, narrow instrument. When mites are found they may be aspirated into a glass vial made into an aspirator (Fig. 1). Under experimental conditions, after mites had been starved for 48 hours, the time required for them to feed and drop from a host

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varied from 10 minutes to 120 hours with the majority leaving the animals between 6 and 8 hours after introduction. A rat nest may be examined in a manner similar to the examination of the wood shavings.

Utilizing the descriptions of the adult mites given by Hirst (1913) and Ewing (1923) one can readily distinguish *L. bacoti* from other mites commonly found on rodents.

FIG. 1. Equipment and procedure for examining wood-shavings for mites.

Large colonies of mites can be successfully reared by creating an artificial nest which approximates a rat nest as found in nature. Such an artificial nest can be constructed by depositing a half-inch layer of loam soil over the bottom of a box, atop several thicknesses of newspaper, over which straw should be placed and bunched against the box sides. The soil readily absorbs urine which may be detrimental to the welfare of the mites while the straw supplies them with a large surface area over which they can easily crawl. Animals in small cages, which can easily be constructed from a roll of one-half-inch-mesh galvanized wire (Fig. 2), may be placed on the straw within the box (Fig. 3) and mites placed on the animals. If, in time, the straw under the cages should become matted, fresh straw may be placed directly on top of the old. The number of animals to be placed in a nest will

determine the size of the box to use. When four or more animals are considered for the same nest a long narrow box was found to be superior to a square one which does not as readily allow for the most satisfactory bunching of the straw around the ends of all cages (Fig. 4).

Sand used in place of soil gave inferior results apparently because the mites found it difficult to crawl over particles which so readily rolled over each other. It was also found that colony development was much slower and irregular when either grass or wood shavings were substituted for straw. Grass has the tendency to mat together too easily and to accumulate and hold urine, whereas straw, which is much

FIG. 2. Cotton rat in easily constructed, one-half-inch-mesh wire cage.

more rigid, does not mat as readily and dries out quickly. When wood shavings were used it appeared that the mites found it difficult to locate the host because of so many independent units over which they had to crawl and among which they could get lost.

Urine should be kept at a minimum. This can best be done by giving the animals water only in their food supply. Strips of water-soaked sweet potato are very satisfactory for this purpose. A minimum atmospheric humidity of 70 per cent should be maintained. More favorable results can be obtained with a relative humidity of from 85 to 90 per cent. Egg production decreases and the time necessary for development increases as the humidity is lowered.

A ring of vaseline placed around the inside edge of each box is useful in preventing mites from leaving the boxes. However, a number do manage to escape and to prevent them from wandering about the room the boxes can be placed on

FIG. 3. A nest for two rats.

specially constructed metal trays, which have built-in troughs around the edges. The troughs may be filled with automobile lubricating oil.

In such an artificial nest as described, a colony, which was started with 32 mites feeding on two rats, was established. Within two weeks the number of mites in this colony had increased to such an extent that only an approximation of their numbers could be made; 1,000 appeared to be a conservative estimate.

At a time when the growth of the colonies was at a maximum, certain observations on the life cycle and development of the tropical rat mite were made. Mites

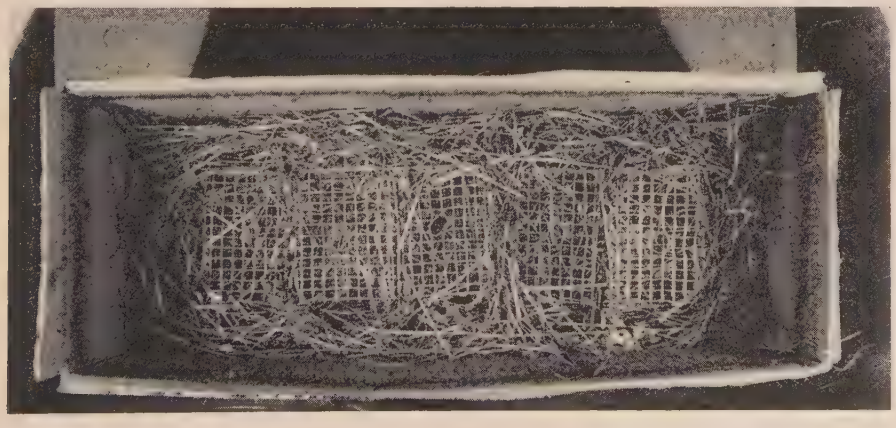

FIG. 4. A nest for five rats.

placed in corked glass vials soon after feeding and kept at a room temperature of approximately 21° C and at an unknown humidity were observed. It was found that the average number of eggs deposited by any given female after a blood meal was 7+ and that another feeding was necessary before more eggs could be produced. About 3 to 5 hours elapsed between the deposition of any two eggs by any given female. The eggs required from 38.5 to 53.5 hours to hatch and 19 to 24 hours after the larvae emerged they cast their skins, without partaking of a blood meal, and an eight-legged nymphal stage appeared. Females probably deposit a group of eggs after each feeding which may take place as often as every second day under the conditions described.

As the number of mites in a colony increase to literally thousands there is danger of the hosts dying of marked anaemia. If the mites cannot be utilized at a rate which approximates the growth of the colony, it is advisable, if the lives of the rats are valued, to remove them from the nests periodically and substitute others or allow the colony to decrease, by the death of its members, for lack of food.

Since the initial colony was started many others have been successfully developed and maintained, utilizing the technique described.

SUMMARY

1. When large populations of the tropical rat mite, *Liponyssus bacoti,* are produced in the laboratory, induced filarial infections among rodents permit investigators of filariasis to undertake research problems which heretofore have been closed.

2. The tropical rat mite can be reared and maintained in the laboratory by constructing artificial rat nests similar to those found in nature. Directions for rearing are given and precautions offered.

3. Phases of the life cycle of *L. bacoti* are mentioned. After a blood meal females deposit an average of 7+ eggs which hatch in 38.5 to 53.5 hours. Nineteen to 24 hours after hatching the larvae can molt without partaking of a blood meal and an eight-legged nymphal stage appears.

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STUDIES ON THE LIFE HISTORY AND DEVELOPMENT OF *MONILIFORMIS DUBIUS* MEYER, 1933[1]

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HISTORICAL

Grassi and Calandruccio (1888) reported the occurrence of *Echinorhynchus moniliformis* (*Moniliformis moniliformis*) in the small intestine of *Mus decumanus* and *Myoxus quercinus* in Sicily. They found the common beetle *Blaps mucronata* to be the intermediate host, and three times the authors found more than one hundred juvenile *E. moniliformis* in the body cavity of a single *Blaps*. They fed several of these juvenile acanthocephalans to a young rat and two weeks later recovered worms 1 cm long. Calandruccio proved the susceptibility of man to infection with this acanthocephalan by ingesting the juveniles recovered from the beetle. Five weeks were required for the worms to reach sexual maturity.

Magalhães (1898) reported the occurrence of the juveniles of *Moniliformis moniliformis* (*M. dubius*) in the body cavity of *Periplaneta americana* collected in Rio de Janeiro. His identification of these larvae was made on the basis of shape, size, and distribution of the proboscis hooks, and was not verified by experimental feeding.

Travassos (1917) found the juveniles of *Moniliformis dubius* (*M. travassosi* Meyer, 1933) in the body cavity of *Periplaneta americana* in Brazil.

In preliminary attempts to infect cockroaches with *Moniliformis dubius,* Chandler (1921) reported negative results. However, in later work, investigators in Chandler's laboratory (Chandler, 1941) have had no difficulty in infecting *P. americana* with *M. dubius.*

Southwell (1922) found specimens of *M. moniliformis* (*M. dubius*) encysted in the body cavity of *P. americana* in West Africa.

Sandground (1926) found numerous small ACANTHOCEPHALA in the intestine of two specimens of *Bufo marinus* and in a single specimen of the lacertilian genus *Ctenosaura,* and identified them as the post-cystic stages of *Moniliformis moniliformis.* Chandler (1941) states that these specimens probably belong to a new species, the adults of which have not been described.

Although the intermediate host of *Moniliformis dubius* has been known for some time and the acanthella described more or less fully, no information concerning the developmental cycle in the intermediate host is available in published form except the general discussion of the cycle by Burlingame and Chandler (1941).

MATERIALS AND METHODS

The particular strain of *Moniliformis dubius* used in these experiments was obtained from naturally infected *Periplaneta americana* collected from the Houston Zoological Gardens, where wild rats are abundant and have a high incidence of in-

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[1] A contribution from the Department of Biology, The Rice Institute, Houston, Texas.

[2] The author wishes to express his appreciation to Professor Asa C. Chandler, under whose direction this investigation was made, for his inspiration, encouragement and many valuable suggestions.

fection with *Moniliformis dubius*. The percentage of infection in the roaches was between 80% and 90% at the time the original stock of infected roaches was collected (October, 1939). For recovery of the encysted acanthellas, roaches were etherized, the head, wings and legs trimmed off, a median dorsal longitudinal incision made in the abdomen, and the contents of the body cavity washed out into a Petri dish with a pipette filled with psysiological saline. By this method the encysted acanthellas were usually obtained free of debris and adhering adipose tissue.

As experimental final hosts, three months old, laboratory-reared, helminth-free albino rats were used. The acanthellas were administered orally by means of a pipette. The experimental rats were kept in a roach-proof enclosure to prevent the spread of the infection to the rest of the rat colony. Fecal examinations were begun five weeks after infection. The wet smear technique and concentration by centrifugation were used for the fecal examinations, since ACANTHOCEPHALA eggs cannot be recovered by the flotation technique.

The roaches used for experimental intermediate host infections were obtained from the basement of the Physics Laboratory building at the Rice Institute, and were known to be free of ACANTHOCEPHALA infections as a result of numerous periodical examinations. Stock cultures of infected and uninfected roaches were maintained in large glass aquaria with a half inch layer of moist sand in the bottom and several pieces of moistened paper towel crumpled on the sand to maintain a high humidity and to provide shelter. The tops of the aquaria were covered with glass. The roaches were fed on Pablum.

For the experimental infection of the roaches, eggs from the body cavity of mature female worms were used. The worms were removed from the small intestine of the rats and placed in physiological saline. The posterior extremity of the female worms was cut off and the contents of the body cavity stripped into a centrifuge tube. This material, consisting of egg balls and immature and mature eggs, was then washed thoroughly by centrifuging in several changes of tap water. The ACANTHOCEPHALA-free roaches, isolated in pint fruit jars with screen tops, and kept without food for several days, were infected by feeding Pablum to which a few drops of washed egg suspension were added. As soon as each roach had eaten all of the contaminated Pablum it was removed to the experimental culture jar where groups of experimental roaches were kept.

MICROSCOPICAL TECHNIQUE

Adult and larval ACANTHOCEPHALA were removed from their respective hosts in physiological saline. In order to bring about the evagination of the proboscis of the adults and infective larvae without mechanical pressure, which may distort the hook pattern, they were placed in either tap water or distilled water. When this method fails, as it does with certain specimens, mechanical pressure must be used. Bouin's Picro-Formol fixative used at room temperature gives very satisfactory fixation for larval and adult ACANTHOCEPHALA as well as for tissues containing the larval forms.

Mayer's Carmalum diluted to a 1.5–2% solution with 0.02–0.03 cc glacial acetic acid added to each 50 cc of stain was found to be the most satisfactory stain for toto mounts. Carmalum brings about a clear, permanent nuclear differentiation essential for the morphological studies.

After destaining with 70% alcohol, a saturated solution of Indigo-Carmine in

95% alcohol was used for staining the hooks. The staining time varies from a few minutes to several hours in different specimens. Care must be taken not to leave the specimens in the Indigo-Carmine too long because the stain will eventually penetrate the cuticle and mask the Carmalum.

In connection with the study of ACANTHOCEPHALA life histories, careful observations of the changes in the acanthor as it passes through the gut wall of the intermediate host are necessary. Toto mounts of the gut wall containing the larvae are unsatisfactory since the staining of the gut tissue masks the structure of the acanthor, and since it is often impractical to make dissections at this stage, serial sections of the gut are necessary. The gut is removed in physiological saline, most of the malpighian tubules trimmed off, and the contents flushed out with saline. It is then slit open, stretched out on a slide, and flattened slightly by a cover glass. Microscopic examination will reveal whether or not the acanthors are numerous enough for making sections. If the gut is to be sectioned, it is fixed while still under the cover glass with Bouin's Picro-Formol fixative. It is then cut into small squares (about 5 mm) and all the pieces from one gut imbedded in the same paraffin block, from which sections 5–10 μ are cut.

For sectioning larvae that had become free in the body cavity, the method described by Davies (1929) for orienting embryological material for sectioning was used with slight modifications. Weigert's Iron Hematoxylin was used to stain all sections. This stain seems to have a definite affinity for the large nuclei characteristic of the ACANTHOCEPHALA and very little affinity for the surrounding parenchymatous tissue.

For detailed study of the acanthor stage the technique described by Manter (1928) with the improvements described by Moore (1942) was used.

TERMINOLOGY

Prior to 1935 no attempt had been made to apply an exact terminology to larval stages in ACANTHOCEPHALA life cycles. Van Cleave (1935 and 1937) recognized the existence of at least two distinct larval stages in acanthocephalan life histories. He proposed the name "acanthor" to designate the larval form which emerges from the egg. This stage varies in external appearance and internal organization among the various genera, and it is possible that when more complete descriptions of the acanthors are available, they may throw light on phylogenetic relationships.

Van Cleave proposed the term "acanthella" to designate the series of immature stages of an acanthocephalan which develop progressively within the body cavity of the invertebrate host. During this developmental period the anterior spines of the acanthor are lost and the internal organization of the adult worm is laid down by differentiation of the central nuclear mass which is characteristic of the acanthor stage. Kates (1943) in describing the development of *Macracanthorhynchus hirudinaceus* divides the acanthor stage into two substages and the acanthella stage into six substages. These subdivisions are based on the general morphology and the age of the larvae.

According to Van Cleave's designation the term "acanthella" is a blanket term covering several weeks' development and at least two distinct stages in the developmental cycle.

The author feels that the term acanthella should be restricted to the infective

stage, and should be defined as follows: infective larva of an acanthocephalan, according to existing knowledge is found encysted in the body cavity of the first intermediate host (an invertebrate) or encysted in the mesenteries or other tissues of a transport host (usually a vertebrate). In this stage the proboscis has reached its full development and is characteristically invaginated within the proboscis receptacle. In the invertebrate host the larva is enveloped in a hyaline cyst or sheath which is laid down by the worm. The size and shape of the cyst or sheath varies according to the species. In the transport host the acanthella is encapsulated by closely adhering host tissue.

A short time before the appearance of the acanthella a distinctive stage may be observed in the developmental cycle, for which the term "pre-acanthella" is proposed. The author has observed this stage in the life cycle of four species of ACANTHOCEPHALA worked on by him and has noted that previous descriptions of acanthocephalan life histories usually illustrate this stage. The following general characterization of the pre-acanthella is given: elongate in form, and immobile; body shape may be straight, slightly curved or bent back upon itself; proboscis usually projecting straight forward. The definitive proboscis pattern is laid down, but the developing proboscis hooks have not broken through the cuticle. The retractor and inverter muscles of the proboscis and proboscis receptacle are fully developed but are not functional. The body cavity and cavities within the proboscis are fully developed and will only be changed in shape by additional growth. The reproductive organs are recognizable. The hypodermis possesses many large vesicular nuclei with no discriminate arrangement. The lemniscal ring of nuclei is in evidence. An apical ring of nuclei may be found at the tip of the developing proboscis. The body is enveloped in a closely adherent hyaline sheath and as far as is known is found in the body cavity of the first intermediate host.

GENERAL LIFE HISTORY OF *Moniliformis dubius*

The fully embryonated eggs, passed by the female worm, hatch in the mid-gut of the cockroach, *Periplaneta americana,* twenty-four to forty-eight hours after ingestion. By the end of twenty-four hours the outer shell of the egg is lost and the movement of the acanthor within the confining inner shell can be observed. By the end of forty-eight hours the acanthors have freed themselves from the confining limits of the inner egg membrane and may be found free in the lumen of the gut, or actively penetrating the gut wall by retraction and extension of the rostellar hooks. During migration through the tissues of the gut wall the acanthors do considerable wandering. In about ten to twelve days after infection the larvae appear as minute iridescent specks on the outer wall of the gut. A day or so later these larvae drop into the cavity, where they lie free or become embedded in the adipose tissue. Here in the body cavity, progressive development occurs whereby the definitive organization of the mature worm is laid down by the differentiation of the central nuclear mass. The pre-acanthella stage appears from five to six weeks after infection. Complete development to the encysted acanthella requires from seven to eight weeks, depending upon the environmental temperature of the intermediate host. In the author's experiments the temperature within the roach culture jars ranged from 23° to 26° C. After development is completed the encysted acanthellas lie dormant in the body cavity of the roach until ingested by a suitable final host.

In the final host the cyst enveloping the acanthella is dissolved in the stomach, and the acanthella stimulated to evert its proboscis. Upon passage into the small intestine the acanthella attaches itself to the intestinal wall without a preliminary migratory phase. The prepatent period in the final host ranges from five to six weeks as shown by Burlingame and Chandler (1941).

DESCRIPTION OF DEVELOPMENTAL STAGES

Egg (Fig. 1)

The egg is elliptical in shape and provided with two distinct shells plus two adhering membranes. The clear thick outer shell is covered by a very thin wrinkled membrane which is easily lost. This outer shell is smooth in outline, and in spite of its thickness is not particularly rigid in structure. Just beneath the outer shell lies the inner shell or middle embryonic envelope, which is rigid in structure and highly refractive. Between the middle embryonic envelope and the acanthor is a thin elastic membrane which is usually closely adherent to the acanthor, which has its rostellar hooks invaginated. Yamaguti and Miyata (1938) refer to an inner shell as well as outer and middle ones, but their photomicrograph shows no such structure. Possibly these authors were working with preserved eggs in which the acanthor had shrunk away from the thin membrane which is closely adherent to it in life.

There has been some disunity in descriptions and measurements of the eggs of *M. dubius* probably due to differences in source, and treatment prior to measuring. The eggs were observed to undergo some distortion and shrinkage when subjected to fixing and dehydrating agents, and a slight swelling of the outer shell when the eggs were left standing in water for several days.

The writer's measurements, made on fresh, viable, fully mature eggs, either from the body cavity of female worms or in feces of infected rats were as follows: outer shell 112 to 138 μ by 54 to 64 μ, with a thickness of 12 to 15 μ at the anterior pole and 8 to 9 μ at a midpoint on the lateral surface; middle embryonic envelope 83 to 100 μ by 38 to 41 μ with a thickness of 3 to 4 μ; enclosed acanthor 77 to 93 μ by 32 to 35 μ.

Acanthor (Figs. 2 & 3)

The artificially hatched acanthor measures 145 to 154 μ by 41 to 42 μ, the width being measured at a mid-point between the posterior limit of the rostellum and the posterior end of the body. The rostellum is wider than it is long, and measures 30 to 32 μ by 56 to 58 μ.

The rostellum is armed with six blade-like hooks, one medial pair, two lateral pairs, and an additional two circular rows of rostellar spines. The two median rostellar hooks originate at the apex of the rostellum and extend outward, being connected to the rostellum except for about 3 μ at the tip. They measure 18 to 19 μ in length and are 4 μ wide at the widest point, which is about 3 μ from the tip of the hook. The lateral hooks are located one on either side of each median hook, and they measure 15 to 18 μ by 2 to 3 μ. The first circular row of rostellar spines consists of 10 or occasionally 12 spines arranged as follows: three, occasionally four, on either side of the rostellum between the lateral hooks of opposite sides, and one between the median hook and each lateral hook on either side. These spines take

root at a point just posterior to the tip of the median hooks. They measure 9 to 12 μ in length. The second circular row is usually composed of 12 to 14 spines 6 to 9 μ in length. The rostellar spines grade into the minute cuticular spines of the body, which are arranged in alternating circular rows of 12 to 16 spines in each row near the anterior end of the body and 6 to 8 spines in each row near the posterior end.

The musculature of the acanthor consists of two retractor muscles and a circular muscular band associated with the rostellum. The retractor muscles are inserted together in the center of the rostellum between the two median hooks, and originate at a point on the body wall about one third of the way back toward the posterior end. The circular muscular band of the rostellum is located just beneath the cuticula. The body wall of the acanthor is provided with subcuticular muscles which are made up of a network of thin muscle fibers running in a clock-wise spiral.

The central nuclear mass of the acanthor consists of a band of closely packed, undifferentiated nuclei from which the structure of the ensuing larval stages will develop. This mass of nuclei is located in the center of the acanthor, beginning at the base of the rostellum and extending about two-thirds of the body length. The concentration of nuclei is greatest at the anterior end. There are a few scattered vesicular nuclei located beneath the network of subcuticular muscles. It is assumed that these nuclei have differentiated from the central nuclear mass while the acanthor was confined within the egg membranes, and will serve as functional nuclei for the duration of the acanthor stage.

Changes Taking Place in the Development from the Acanthor to the Pre-acanthella (Figs. 4 & 5)

As the acanthor progresses through the tissue of the intestinal wall of the intermediate host it loses its characteristic spindle shape and becomes oval, gradually losing its motility so that when the larva reaches the exterior of the gut it is entirely quiescent. As stated previously, the larvae appear on the exterior of the gut wall 10 to 12 days after infection. At this period the developing larvae measure 142 to 193 μ by 90 to 167 μ. All measurements were made from stained toto mounts unless otherwise stated. The larva clinging to the exterior of the gut wall is composed of two distinct layers: an outer dermal layer and an inner nuclear mass. The dermal layer is composed of loosely granulated parenchyma containing large, scattered, spherical nuclei which have differentiated from the central nuclear mass of the acanthor. The nuclei of the central nuclear mass have become vesicular and are surrounded by definite nuclear membranes.

From 12 to 22 days after infection, the shape of the larva changes from oval to nearly spherical. The larvae remain spherical for a short time and then begin to elongate. During this period of development they measure 182 to 350 μ by 140 to 308 μ. The larvae recovered from any one roach will not be of uniform size nor will they be in the same stage of development. This variation in size and rate of development may be attributed to several factors: (1) variation in the time required for hatching in the gut of the roach, (2) length of time required to penetrate the gut wall, (3) abundance of food material available in the portion of the body cavity where the larva is located, (4) individual differences of larvae as to their ability to assimilate available nourishment.

The rostellar hooks characteristic of the acanthor stage are still in evidence at the

anterior end of the larva, but their position and relationship to each other are greatly altered due to the growth of the larva. Very little change has occurred in the appearance of the dermal layer except that the large spherical nuclei have become more numerous. Considerable change has taken place in the inner nuclear mass, since at this time the primordia of various adult structures can be observed. Most obvious of these is the primordium of the brain. It appears as a rounded mass of finely granular parenchyma-like tissue containing only 5 or 6 nuclei at first. As the larva grows these nuclei divide and the primordium of the brain soon becomes a compact mass of small nuclei. The primordium of the proboscis is first observed as an elongate mass of nuclei anterior to the brain. Nuclei in close proximity to the brain soon arrange themselves to form the primordium of the proboscis receptacle, which encloses the brain and extends anteriorly to connect with the proboscis primordium. Posterior to the brain and proboscis receptacle, a median longitudinal split may be observed in the inner nuclear mass; this is the beginning of the body cavity of the adult worm.

From 22 to 29 days after hatching, the larvae (Fig. 4) elongate considerably. Larvae removed from the body cavity of the roach on the 29th day measured 322 to 518 μ by 210 to 280 μ. The developing proboscis has become a solid cylinder of tissue separated from the dermal layer except at the anterior end. The developing proboscis hooks are observed at this stage as irregularly hexagonal areas which appear as hollow spaces in sagittal section. The proboscis receptacle is well defined and has become separated from the dermal layer. The body cavity extends nearly to the posterior end of the larva. At the posterior end of the body cavity there is a solid aggregation of nuclei from which the genital primordia will arise. The internal periphery of the body cavity is lined by several irregular layers of nuclei which later become incorporated into the body musculature. The developing lacunar system is observed as a series of transverse striations encircling the larvae in the dermal layer immediately outside of the nuclei lining the body cavity. Little change is observed in the dermal layer except that the migration of nuclei into it from the central nuclear mass has ceased and the nuclei are more widely dispersed.

During the period of 30 to 38 days after infection the larvae continue to increase in length. By the end of 38 days of development most of the larvae measure 548 to 640 μ by 182 μ. The developing proboscis hooks have grown out toward the dermal layer. The retractor and inverter muscles of the proboscis have differentiated from the tissue within the limits of the receptacle. In the sectioned larva illustrated (Fig. 5), one retractor muscle can be seen extending from the receptacle to its origin in the body wall. The genital ligament extending from the posterior end of the larva to the base of the proboscis receptacle is evident. The primordia of the genital organs are more distinct in the mass of tissue in the posterior region of the body cavity. The larva figured was destined to become a male as shown by the indistinct outline of the testes in the genital primordia. In the female the ovaries develop from cells contained in the genital ligament. At this stage in development the thin hyaline sheath may be seen in living material closely adherent to the larva. The sheath contains some evidence of cellular structure and is laid down by the larva.

Pre-acanthella (Figs. 6 & 7)

The pre-acanthella usually appears between 38 and 44 days after infection and may measure from 0.96 to 2.25 mm by 0.3 mm. In addition to the overall length and

breadth the following measurements were taken: Dermal layer in mid-region of body, 0.07 to 0.12 mm thick; width of body in region of lemniscal ring, 0.16 to 0.22 mm; combined length of proboscis and proboscis receptacle, 0.30 to 0.46 mm; length of proboscis anterior to lemniscal ring, 0.19 to 0.5 mm.

The central portion of the proboscis has become hollow and the inverters pass through the center of the hollow proboscis, inserting at the anterior tip. Posteriorly the inverters pass around the brain on opposite sides, where a portion of each band becomes fused with the postero-lateral wall of the receptacle. The remainder of each band passes through the wall to become the retractor muscles of the proboscis. The retractors extend posteriorly and fuse with the body wall. Each inverter muscle contains 2 or 3 small nuclei and each retractor muscle contain 3 or 4 larger nuclei.

The hooks of the proboscis appear as thin, highly refractile lines extending through the hypodermis, but remaining beneath the cuticula. Small roots can be seen at the bases of the hooks, which are arranged in definite longitudinal rows usually with 10 or 11 hooks in each row. The hooks have not become recurved as yet but are practically straight with only a slight posteriorly-directed curvature.

The lemniscal ring is composed of 13 or 14 hypodermal nuclei which have migrated to the base of the proboscis. The other hypodermal nuclei are confined to the region posterior to the lemniscal ring except 6 smaller nuclei which have arranged themselves at the tip of the proboscis as the apical ring.

In male specimens the immature testes may be seen arranged in tandem in the posterior region of the body cavity, and the primordia of the rest of the male genital structures are located in the posterior tip of the body cavity. In female specimens the uterine bell assumes a similar position.

The rostellar hooks of the acanthor are sometimes observed still attached to the cuticula as late as the pre-acanthella stage (Fig. 7). These hooks have become so dislocated by the growth of the larva that they may be located as far posterior as the insertion of the retractor muscles.

The pre-acanthella is usually confined within a clear hyaline sheath, and is often bent double within that sheath.

Acanthella (Figs. 8, 9, & 10)

From the 44th to 51st days in the developmental cycle, the larva continues to elongate, the proboscis becomes more distinctly set off from the body, and the lemniscal buds begin to develop. By means of these changes the pre-acanthella begins the change into the acanthella. Fifty-one days after infection the larvae measure 2.51 mm to 2.61 mm in length. The hypodermis, which up to this time has been quite narrow, begins to expand laterally in the anterior region of the body, causing the larva to lose its cylindrical shape and become more or less flattened. The nuclei of the hypodermis are round and vesicular as they were in the pre-acanthella, which is an indication that the larva has not reached the infective stage. The proboscis of this non-infective stage is 0.42 mm long, 0.09 to 0.12 mm wide at the tip near the first row of hooks, 0.16 to 0.8 mm wide near the middle, and 0.11 to 0.14 mm wide at the base. The hooks of the proboscis have become thicker, recurved, and more heavily chitinized, but have not as yet broken through the cuticula. Each hook pushes against the cuticula so as to form rows of small protuberences on

the surface of the proboscis. At this stage the proboscis remains extended, since its musculature has not become functional.

Two short curved retinacula may be observed extending from the base of the proboscis receptacle. The origin of the retinacula is not known. In the pre-acanthellas examined by the author none could be found, but they were visible in most of the 51-day-old larvae and in some of the acanthellas.

The lemnisci develop as two evaginations of the hypodermal layer into the body cavity at a point slightly anterior to the brain. The nuclei of the lemniscal ring migrate into these buds soon after they develop. The nuclei may be distributed in the lemniscal buds as follows: 7–6, 7–7, 8–6. Kates (1943) has shown that in the development of *Macracanthorhynchus hirudinaceus* there are 12 nuclei in the lemniscal ring and that six nuclei migrate into each lemniscus.

Development takes place rapidly at this point in the life cycle and in a few days the acanthella or infective stage is reached, requiring a total of 49 to 55 days from the time the eggs are ingested by the intermediate host. The infective acanthella can be differentiated from the non-infective stage, looking very much like it, by the position of the proboscis and condition of the hooks. If the proboscis is invaginated within the body cavity of the living acanthella (Fig. 9) and the tips of the hooks have broken through the cuticula, the acanthella may be considered infective (Figs. 8, 10). The acanthella has been described and illustrated by previous investigators since it is this stage that has been found in the body cavity of the intermediate hosts. Bacigalupo (1927) figured several photomicrographs of the acanthella. Burlingame and Chandler (1941) showed an excellent photomicrograph of a stained acanthella.

The acanthella is enclosed in an ellipsoid hyaline cyst measuring 1.5 to 1.8 mm by 0.90 to 0.95 mm. The acanthella (proboscis invaginated) within the cyst measures 1.05 to 1.3 mm by 0.79 to 0.84 mm (measurements made from living material). Fixed and stained specimens with the proboscis fully extended measure 2.28 to 2.37 mm in length. The body measures 0.64 to 0.91 mm in width at its widest point, which is near the anterior end. The width of the hypodermis in this area ranges from 0.22 to 0.73 mm depending upon the degree of longitudinal contraction of the acanthella.

The reduction in length of the larva from the pre-acanthella stage to the acanthella stage is due to the development of the thick flange-like lateral fields of the hypodermis. In fresh material the nuclei of the hypodermis appear as irregularly arranged highly refractile spots. In stained specimens these nuclei are very irregular in outline, and surrounded by clear areas. They appear to have undergone partial dissolution, a process which eventually becomes complete.

The body of the acanthella is widest near the anterior end, tapering with more or less uniformity to the narrow posterior end. The body cavity is vase-shaped with the widest part located near the base of the proboscis receptacle. The main longitudinal vessels of the lacunar system are located laterally in the walls of the body cavity, and are connected by a series of very fine transverse tubules which appear as striations surrounding the body cavity. A series of fine tubules, also emanating from the longitudinal tubes, extends into the lateral fields of the hypodermis for a short distance.

The proboscis and receptacle have reached their full development in the acanthella, and since there is no essential difference between the proboscis of the

acanthella and that of the adult worm, the description of the proboscis and hook pattern will be omitted.

The lemnisci are quite long, and their nuclei are more or less evenly distributed throughout their length. The lemnisci do not extend directly posteriorly but are usually twisted about each other and folded back on themselves. Measurements of the lemnisci are impractical because of their contractility, which renders their length variable both in fresh and fixed specimens.

The sex of the acanthella is easily determined even in living specimens. The testes are subovate in shape and measure 82 to 84 μ by 42 to 48 μ. They are located in tandem in the narrow posterior region of the body cavity. A single vas deferens formed by the union of two short vasa efferentia passes into the eight cement glands which are very small and undeveloped in the acanthella. The vas deferens then joins the seminal vesicle, which communicates to the exterior via the ductus ejaculatorius. No compact ovary is present in the female, but small compact masses of cells, which are immature egg balls, may be seen free in the body cavity or in the genital ligament.

INFECTION OF THE FINAL HOST

A few experiments were performed to study the changes which occur when the acanthella reaches the intestine of the final host. Rat 802 was given 40 acanthellas orally. The rat was killed and autopsied 41½ hours later and 31 worms were recovered. These worms had grown about 0.3 mm during the 41½ hours that they had been in the final host. Twenty-six of these worms recovered from Rat 802 were fed to Rat 804 to see if they were able to become established in the intestine of a new rat. Rat 804 was killed and autopsied three weeks later and only one worm was recovered. Apparently the other twenty-five immature worms were unable to resist the gastric juices of the rat. The development in the final host is one of growth in length, development of the hypodermis into the body wall of the adult worm, and the maturation of the reproductive organs. The prepatent period in the final host, as shown by Burlingame and Chandler (1941) ranges from five to six weeks.

Acanthellas of *Moniliformis dubius* were fed to guinea pigs and rabbits but no infection could be established in these animals.

One cotton rat (*Sigmodon hispidus*) was given 10 acanthellas of *M. dubius*. The animal was autopsied 3½ months later and one female worm recovered. The worm occupied nearly all of the available space in the lumen of the small intestine throughout its length. The eggs in the body cavity of the worm were fertile, indicating the previous presence of at least one mature male worm. The cotton rat constitutes a new experimental host for *M. dubius*.

BEHAVIOR IN COLD-BLOODED VERTEBRATES

Sandground (1926) reported the presence of larval *Moniliformis* attached to the intestinal wall of toads and lizards in Central America, and considered them accidental parasites in these hosts. Chandler (1941), however, failed to obtain attachment when acanthellas of *M. dubius* were fed to toads. He suggested that the toads and lizards found harboring *Moniliformis* in Central America might play more than an accidental rôle in the life cycle of some previously undescribed species of the genus.

To get further information on the possible rôle of cold-blooded vertebrates as transport hosts for *M. dubius,* the writer fed experimentally infected roaches con-

taining infective acanthellas of *M. dubius* to two frogs (*Rana pipiens*), one hog-nosed snake (*Heterodon contortrix*), and to three toads (*Bufo valliceps*).

No ACANTHOCEPHALA were found in either of two frogs when they were autopsied after five and ten days respectively. Several dead acanthellas were found in a dried pellet passed by the hog-nosed snake, their death probably being attributed to desiccation. No ACANTHOCEPHALA were found when the snake was autopsied at the end of a month. Of the three toads, one was negative when autopsied at the end of eight days. Another, autopsied at the end of ten days, still contained undigested chitinous remains of the roach fed to it, apparently not having defecated during this period. One unattached living acanthella was found in the large intestine. The third toad passed a pellet on the tenth day containing remains of the roach fed to it. This pellet contained 24 living acanthellas free of their enveloping cysts but in most cases with the proboscis invaginated. These 24 acanthellas were fed to an albino rat to determine whether they were still infective. The rat was autopsied six months later and four female *M. dubius* were recovered.

It appears, therefore, that acanthellas of *M. dubius* may survive for at least ten days in the alimentary canal of cold-blooded vertebrates which ingest them, without losing their infectivity, but fail to become attached or to penetrate into the tissues. These vertebrates cannot, therefore, be considered as true transport hosts for *M. dubius,* as are armadillos and turkeys for *Oncicola,* and it is obvious that they play no significant rôle in the life cycle of this parasite.

SUMMARY

The terminology used for the developmental stages in ACANTHOCEPHALA life histories is revised by restriction of the term acanthella to the infective stage. The term pre-acanthella is proposed for the elongate stage which appears prior to the acanthella.

The life history and development of *Moniliformis dubius* was studied in natural and experimental infections. The eggs, ingested by *Periplaneta americana,* hatch in the mid-gut. The released acanthor penetrates the gut wall, passes through it in the course of ten to twelve days, drops into the body cavity, and there continues its development. The pre-acanthella appears at the end of 38 to 44 days. The infective acanthella appears about seven to eight weeks after infection. The prepatent period in the final host ranges from five to six weeks.

A new definitive host for *M. dubius* was found by the experimental infection of the cotton rat, *Sigmodon hispidus,* but experimental infections failed in guinea pigs and rabbits. Infective acanthellas, experimentally fed to cold-blooded vertebrates with the intermediate host remain alive and infective for a number of days but fail to attach to the intestinal wall or to penetrate the tissues.

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Explanation of Plates

All figures were drawn with the aid of a camera lucida

Abbreviations Used

B—brain or ganglion
C—cyst
CG—cement glands
CNM—central nuclear mass
CS—cuticular spines
DL—dermal layer
GL—genital ligament
GP—genital primordia
HD—hypodermis
I—inverters
IS—inner shell
L—lemnisci
N—giant nuclei of dermal layer
NA—nuclei of apical ring
NL—nuclei of lemniscal ring
OS—outer shell
P—proboscis
PH—developing proboscis hooks
PR—proboscis receptacle
RH—rostellar hooks
RM—retractor muscles
RS—rostellar spines
T—testes
UB—uterine bell

Plate I

Fig. 1. Mature egg containing acanthor.

Fig. 2. Acanthor in process of escaping from egg shells and membranes. (Drawn from living material.)

Fig. 3. Acanthor dissected from gut wall tissue of *Periplaneta americana* five days after infection.

Fig. 4. Median sagittal section of a larva removed from the body cavity of *P. americana* 29 days after infection.

Fig. 5. Median sagittal section of a larva removed from the body cavity of *P. americana* 38 days after infection.

Fig. 6. Larva removed from the body cavity of *P. americana* 42 days after infection. (This larva is differentiated from the true pre-acanthella of Fig. 7 by the lack of development in the proboscis region and the ratio of body width to body length.)

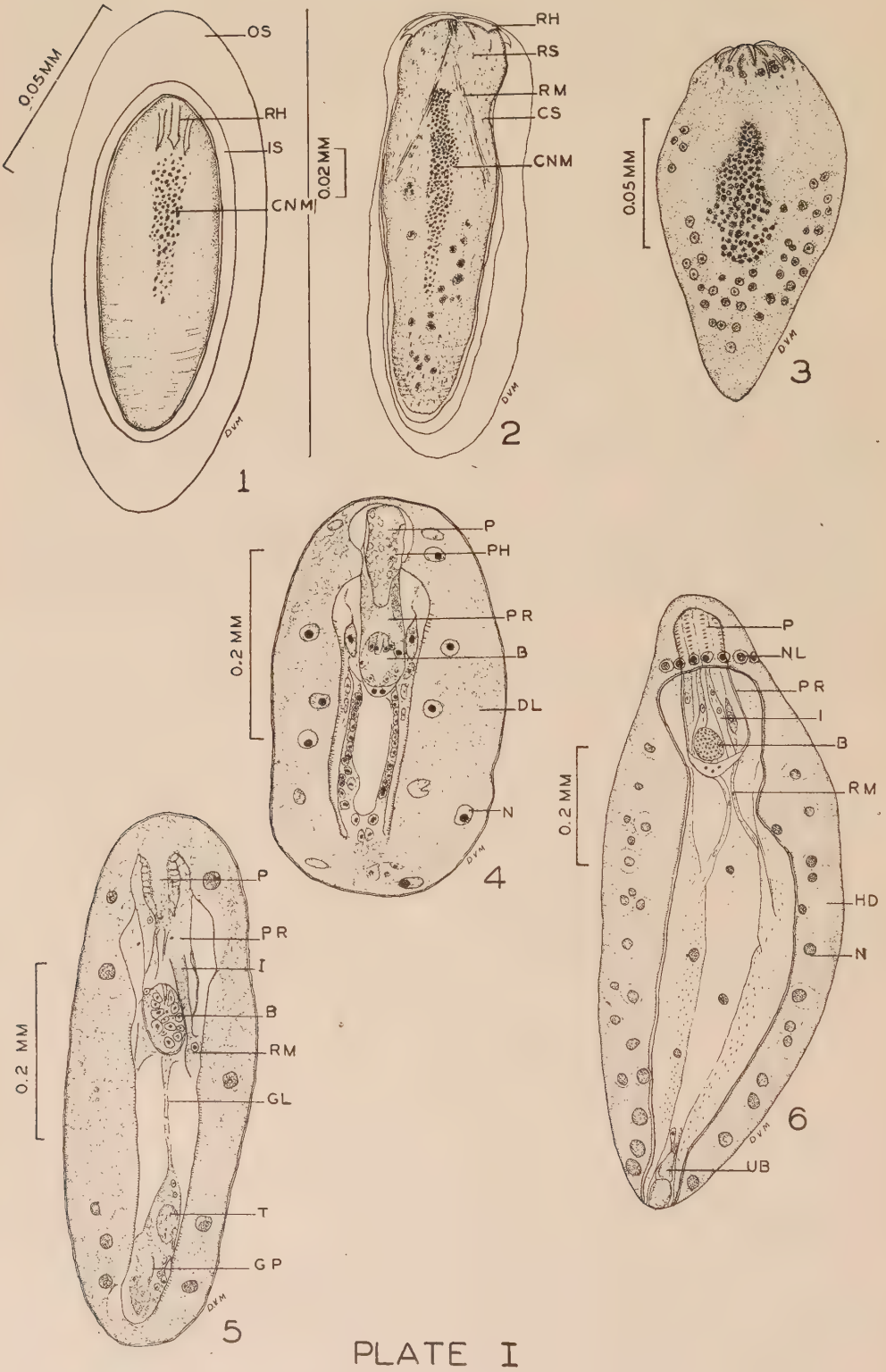
Plate II

Fig. 7. Pre-acanthella dissected from its enveloping sheath. (From body cavity of *P. americana.*)

Fig. 8. Acanthella freed from its cyst and with proboscis evaginated. (From body cavity of *P. americana.*)

Fig. 9. Encysted acanthella from body cavity of *P. americana* with proboscis invaginated. (Drawn from living material.)

Fig. 10. Proboscis of acanthella.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3
0.2 MM
P
PH
PR
B
DL
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4
0.2 MM
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NL
PR
I
B
RM
HD
N
UB
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6
0.2 MM
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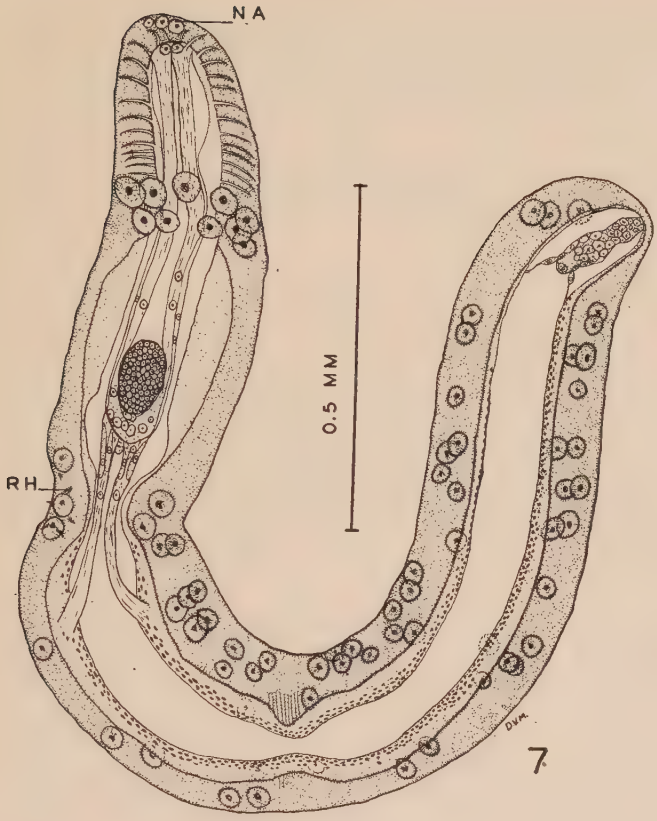
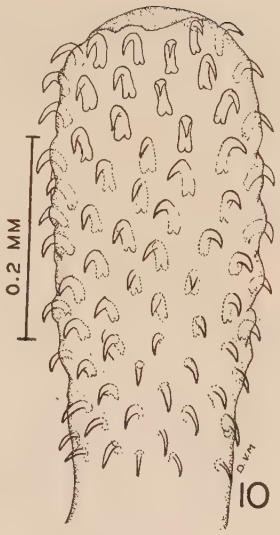
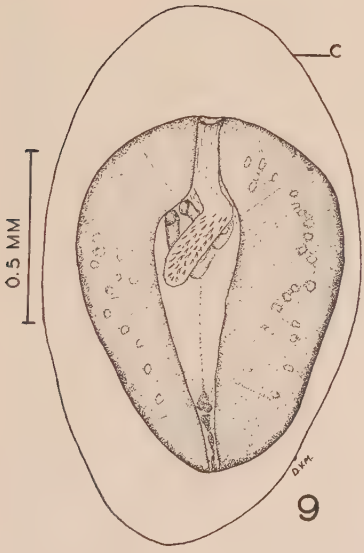
DVM
5

PLATE I


NA
RH
0.5 MM
DVM
7


0.2 MM
DVM
10


C
0.5 MM
DVM
9

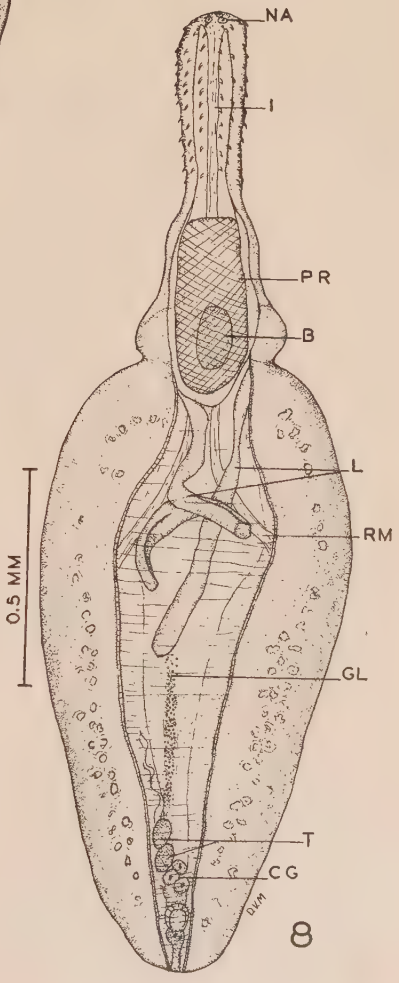

NA
I
PR
B
L
RM
0.5 MM
GL
T
CG
DVM
8

PLATE II

THE MIGRATION OF INFECTIVE LARVAE OF *WUCHERERIA BANCROFTI* WITHIN THE MOSQUITO HOST AND THEIR RATE OF ESCAPE UNDER LABORATORY CONDITIONS

IVAN PRATT AND WALTER L. NEWTON[1]
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INTRODUCTION

The development of *Wuchereria bancrofti* larvae within the mosquito host has been carefully worked out, but the migration of the larvae, once they have become infective, within the mosquito has been a matter of incidental observation. Further, there has apparently been no statistical study to verify an opinion held by some that larvae escape without the mosquito taking additional blood meals. Interest has been focused mainly upon the site of the escape of the larvae from the proboscis.

Francis (1919) observed that the larvae, after a stay of approximately two weeks in the thoracic muscles, migrated to the proboscis. However, in his discussion of the factors limiting the spread of filariasis, he asserted that a large proportion of the larvae after they leave the thoracic muscles migrate into the abdomen and are lost. Yamada (1927) stated that it appeared to be certain that the majority of the larvae first found their way to the abdomen where they seemed to move about in the general body cavity for a short while and then took their course forward, traveling through the thorax and head to the proboscis. Hu (1933) wrote that the distribution of infective larvae within the mosquito suggested that their wanderings did not necessarily take them at once to the head; thus larvae which had escaped from the proboscis would be replaced by others coming up from the thorax and abdomen. Newton and Pratt (1945) demonstrated that infective larvae placed in the abdominal cavity of an uninfected mosquito migrated forward to the proboscis.

Several workers have suggested that infective larvae escape from the mosquito host without the latter taking additional blood meals. Yamada and Komori (1926), while investigating the site of exit of the larvae, found that filarial larvae escaped from the labium by rupturing the tips of the labella when a stimulus such as pressure or heat was experimentally applied. They stated, in addition, that the larvae may also escape spontaneously in the same way when their hosts are fed on sugar-water solution. Hu (1935) examined 22 mosquitoes that had lived from 10 to 93 days following infection. He found infections which had lasted for a considerable length of time, even as long as 79 days after the incubation period of two weeks. However, he noted that his specimens kept after two weeks were held at a lower temperature and a lower relative humidity than those of the first two weeks. Furthermore, the 7 specimens examined through the first 16 days averaged 10 larvae per mosquito, whereas the 15 examined after this time averaged 3 larvae per mosquito; this indicated a definite loss. Galliard (1941) stated that at temperatures between 30 and 35° C the larvae do not remain in the mosquito longer than 9–10 days after they have reached the infective stage. In one experiment he found that the mosquitoes

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[1] The authors wish to express their gratitude to Miss Margaret E. Rice, statistician with the Health and Safety Department, Tennessee Valley Authority, for her valuable assistance in the statistical analysis of the data.

lost their larvae as readily when feeding on sugar water as when feeding on a guinea pig. It was his opinion that large numbers of larvae packed within a proboscis could rupture it, affording exit for the larvae.

There is general agreement that larvae do eventually migrate to the head and proboscis although many of them may remain in the abdomen for variable periods of time. Also, as noted above, there is evidence that larvae can escape from the mosquito host as a result of external stimuli other than those caused by taking a blood meal. However, there has apparently been no statistical or quantitative approach to these subjects, and a study of this sort was undertaken by the writers.

EXPERIMENTAL PROCEDURE

Culex quinquefasciatus females were allowed to feed at night on a patient with microfilariae of the periodic strain of *Wuchereria bancrofti* in his bloodstream. Three lots of mosquitoes were used and while actual numbers were not determined, each lot contained approximately 75–100 females. The latter were given access to the patient's arm between the hours of 11:30 PM and 5:00 AM. Examinations of finger-tip blood between 11:00 PM and 12:00 PM usually revealed 20–30 microfilariae per 20 cubic millimeters.

The mosquitoes which had engorged were placed in cages in an insectary where the temperature varied between 74 and 80 degrees Fahrenheit and the relative humidity between 75 and 85 per cent. The mosquitoes were supplied at all times with sugar-water solution on cotton pads. Previous experiments had shown that in this species the larvae usually attained the infective stage at the end of $16\frac{1}{2}$ days under the conditions described. Beginning with $16\frac{1}{2}$ days and at 2-day intervals thereafter through $30\frac{1}{2}$ days, groups of mosquitoes were killed and each specimen was separated into proboscis, head, thorax, and abdomen. Each portion was dissected in saline and the number of larvae therein was recorded. Examinations were made at $16\frac{1}{2}$, $18\frac{1}{2}$, $20\frac{1}{2}$, $22\frac{1}{2}$, $24\frac{1}{2}$, $26\frac{1}{2}$, $28\frac{1}{2}$, $30\frac{1}{2}$, and $39\frac{1}{2}$ days after the mosquitoes had fed on the infected volunteer. During the earlier dissections many third-stage larvae that had not reached the infective stage were encountered in the thorax. If these larvae were normal and motile, they were considered potentially infective organisms and were counted.

Tabulation of the number of larvae found in each part of the body, as well as the total number, supplied data for the demonstration of two points: first, the analysis of the distribution of the larvae within the mosquito provided information regarding the migration of infective larvae and, second, the total number of larvae found per mosquito indicated any decrease in numbers of larvae present during the successive dissection periods.

RESULTS OF DISSECTION

The results of the dissection of 209 mosquitoes, the total from three separate feedings, are shown in Table 1. During the 14-day interval from $16\frac{1}{2}$ to $30\frac{1}{2}$ days after infection there was a decrease in the average number of larvae per mosquito from 48.7 to 8.8. This loss was not statistically significant until after $18\frac{1}{2}$ days. The average number of larvae per mosquito at $20\frac{1}{2}$ days had dropped to 30.0 and 4 days later the average was 13.6. Again after $24\frac{1}{2}$ days the rate of loss was not statistically significant although a slight decrease in the average number

of larvae continued to occur. The 6 mosquitoes which survived 39½ days contained an average of 1.5 larvae.

TABLE 1.—*Number of larvae of* Wuchereria bancrofti *recovered from proboscis, head, thorax, and abdomen of* Culex quinquefasciatus *from 16½ to 39½ days after infection*

Elapsed time since blood meal	Dissected parts	Mean No. larvae	Standard deviations of observations	Standard deviations of means
16½ days	Proboscis	2.3	1.97	
	Head	1.8	1.53	
	Thorax	32.4	21.80	
	Abdomen	12.2	9.58	
	Total (21 mosquitoes)	48.7	25.12	5.48
18½ days	Proboscis	7.2	4.93	
	Head	4.5	4.66	
	Thorax	23.4	23.82	
	Abdomen	8.0	7.27	
	Total (26 mosquitoes)	43.1	26.83	5.26
20½ days	Proboscis	7.1	6.10	
	Head	4.6	4.71	
	Thorax	14.4	12.26	
	Abdomen	3.9	4.63	
	Total (26 mosquitoes)	30.0	24.06	4.72
22½ days	Proboscis	5.3	3.93	
	Head	3.5	2.99	
	Thorax	8.7	7.56	
	Abdomen	2.7	2.16	
	Total (26 mosquitoes)	20.2	14.07	2.76
24½ days	Proboscis	2.8	3.08	
	Head	1.8	2.74	
	Thorax	6.0	5.15	
	Abdomen	3.0	4.02	
	Total (26 mosquitoes)	13.6	11.54	2.26
26½ days	Proboscis	3.5	3.38	
	Head	2.7	3.66	
	Thorax	3.9	3.41	
	Abdomen	1.5	1.76	
	Total (26 mosquitoes)	11.6	8.48	1.66
28½ days	Proboscis	2.8	2.23	
	Head	1.7	1.84	
	Thorax	3.8	3.86	
	Abdomen	1.9	1.55	
	Total (26 mosquitoes)	10.2	7.23	1.42
30½ days	Proboscis	1.7	1.67	
	Head	1.3	1.38	
	Thorax	3.4	4.38	
	Abdomen	2.4	2.87	
	Total (26 mosquitoes)	8.8	7.36	1.44
39½ days	Proboscis	0.0	....	
	Head	0.0	....	
	Thorax	1.0	....	
	Abdomen	0.5	....	
	Total (6 mosquitoes)	1.5	.....	...

The average number of larvae in the thorax dropped from 32.4 at 16½ days to 6.0 at 24½ days. After this time there was a slight decrease in the average until 30½ days when the count was 3.4. Those examined at 39½ days averaged 1.0.

In the abdomen the average number of larvae decreased from 12.2 at 16½ days

to 2.7 at 22½ days. At 24½ days there was a slight rise to 3.0; at 26½ days the average was 1.5; at 28½ days it had risen to 1.9 and at 30½ days to 2.4. There was an average of 0.5 larva in the abdomens of the 6 mosquitoes examined at 39½ days.

At 16½ days there was an average of 2.3 larvae in the proboscis. By 18½ days the average had risen to 7.2, and was 7.1 at 20½ days. There was a subsequent decrease in the number of larvae to 2.8 by 24½ days. Following this, the average rose again to 3.5 at 26½ days; dropped to 2.8 at 28½ days, and to 1.7 at 30½ days. No larvae were found in the proboscides or heads of the specimens examined at 39½ days.

The fluctuations in the average number of larvae recovered from the head throughout the experiment were similar to those of the proboscis. Generally, the actual averages for the head were about two-thirds those of the proboscis.

DISCUSSION

Table 1 shows that there was considerable variation in the numbers of larvae recovered from the mosquitoes. This may be explained by several factors which were practically impossible to control. First, because it was necessary to feed this species of mosquito in complete quietude and darkness, usually while the patient slept, there was no way of determining when the majority of specimens fed in relation to the maximum concentration of microfilariae in the blood. An attempt to offset this was made by dividing each lot that had fed into equal groups and examining one group for each time interval. In this way had one entire lot obtained fewer microfilariae than another lot, the difference would be spread out over the entire dissection period. Second, it was unlikely that mosquitoes would obtain the same number of microfilariae from a blood meal even though they were feeding side by side at the same time. Third, there was evidence of some individual variation in length of time required for the larvae to reach the infective or migratory stage. In some mosquitoes, larvae required longer than 16½ days to reach this stage and in others, some larvae reached the migratory stage before this time. Changes in the location or number of the larvae in these mosquitoes could not occur until 2 to 4 days before or after migration had begun in the majority of the others.

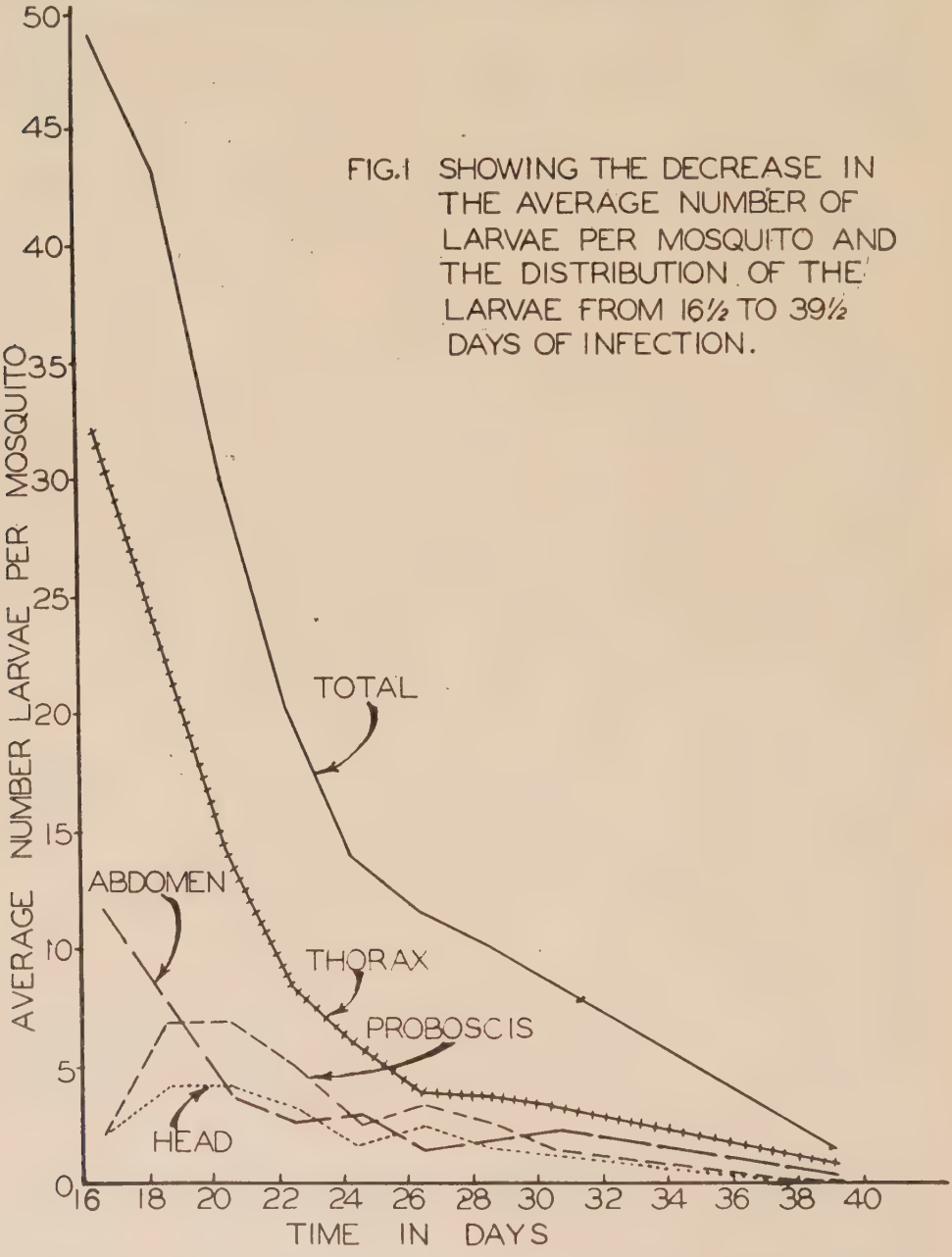
In spite of the variation introduced by these uncontrollable factors, the averages of the total counts in the mosquitoes were found to be stable when statistical analysis was applied.

It is apparent from Fig. 1 that there was a decrease in the average number of larvae per mosquito with each successive time interval although it was most marked between 18½ and 24½ days of infection. Because there was no evidence to indicate that the larvae were dying and being absorbed by the tissues of the mosquito, it must be assumed that the decrease in number of larvae was due to emergence from the mosquito.

The site and time of the emergence of the larvae from the mosquitoes were impossible to observe while the latter were in the cage. However, an anlysis of the dissection results of the various parts of the mosquito at the different time intervals affords an explanation of what was probably taking place.

A comparison (Fig. 1) of these sites with one another at 16½ days reveals that there had been considerable migration from the thorax, where all the larvae were originally undergoing metamorphosis, to the abdomen and, in a lesser degree, to the

head and proboscis. This indicated that the usual migration path after the larvae have become infective is from the thorax to the abdomen. Two days later the thorax was continuing to lose larvae, but they were leaving the abdomen as well and were



migrating up into the head and proboscis. This phenomenon continued for 4 more days, and although the thorax and abdomen were losing larvae at a fairly rapid rate, the head and proboscis were not gaining in actual numbers after 18½ days.

Since the number of larvae in the proboscis was not increasing as a result of the

loss from the thorax and abdomen, it is obvious that larvae were escaping from the mosquito. Assuming that this loss was through the proboscis (the reasons for this

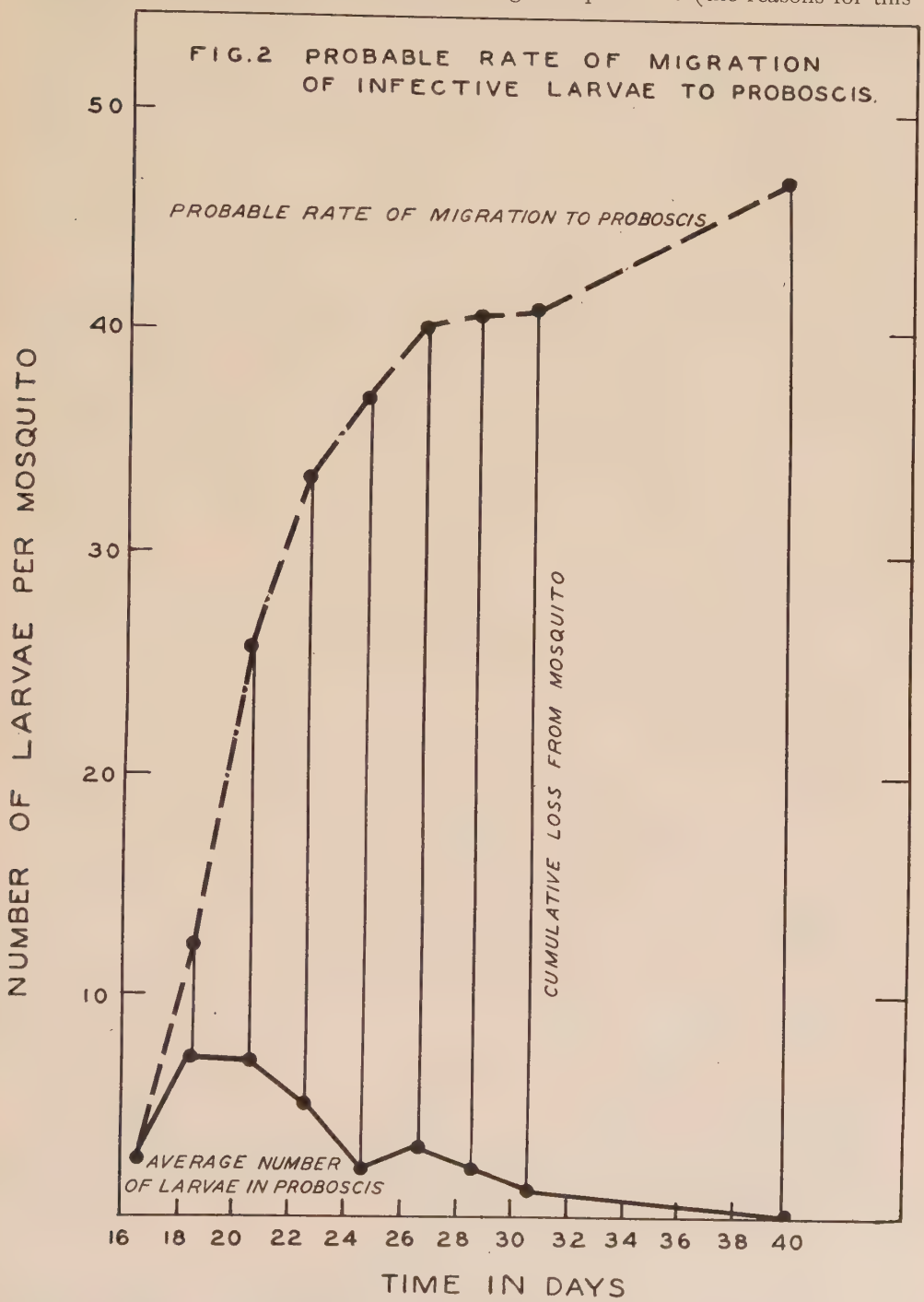


FIG.2 PROBABLE RATE OF MIGRATION OF INFECTIVE LARVAE TO PROBOSCIS.
PROBABLE RATE OF MIGRATION TO PROBOSCIS
CUMULATIVE LOSS FROM MOSQUITO
AVERAGE NUMBER OF LARVAE IN PROBOSCIS
NUMBER OF LARVAE PER MOSQUITO
10
20
30
40
50
16 18 20 22 24 26 28 30 32 34 36 38 40
TIME IN DAYS

conclusion will be discussed later) it follows that the larvae escaping had at one time been in the proboscis. The actual proboscis counts do not, however, indicate

the rate of the migration of the larvae thereto. An estimation of the probable rate of migration to the proboscis was obtained (Fig. 2) by adding the number of larvae found in the proboscis to the number which had been lost by the time of dissection. This loss was ascertained by subtracting the average of the total numbers of larvae found in the mosquitoes at each dissection period from the average total numbers found at $16\frac{1}{2}$ days. Fig. 2 shows that the larvae continued to migrate from the thorax and abdomen to the proboscis at a rapid rate after $18\frac{1}{2}$ days. This continued migration was not revealed by the averages of the proboscis counts shown in the migration curve of Fig. 1.

Referring to Fig. 1 again, there was a statistically significant loss of larvae during the 4-day period from $18\frac{1}{2}$ to $22\frac{1}{2}$ days. After $24\frac{1}{2}$ days there was no significant loss from one dissection period to the next although there was a slight continuing decrease to the end of the experiment. While numbers of larvae in the thorax decreased only slightly, numbers in the head and proboscis as well as those in the abdomen underwent some minor variations; when the head and proboscis averages increased slightly, the abdomen decreased and *vice versa*. This relationship might suggest that the larvae were periodically migrating back and forth from the abdomen to the head and proboscis with an occasional one or two escaping from the latter site. However, this can only be speculated upon because the numbers involved were small, the majority of the larvae having escaped by $24\frac{1}{2}$ days. A variation of two larvae in any of these sites would be sufficient to cause these fluctuations.

The findings indicate that infective larvae escaped throughout the experiment. The cotton pads from which the infected mosquitoes obtained sugar-water during their stay in the cages were examined occasionally. No larvae were recovered. However, observations made during dissections confirm the conclusion that the loss of larvae must have taken place through the proboscis. Direct evidence was obtained when the anterior end of the proboscis was immersed in saline. Larvae emerged from the tips of the labella with no pressure or stimuli other than that exerted by the saline. Spontaneous emergence was found to occur frequently when the proboscis contained 5 or more larvae. Within a minute as many as 8 or 10 would escape completely from the proboscis.

Another observation which suggested the loss of larvae prior to dissection was the finding of many proboscides which appeared to have been ruptured.

The above observations and the fact that larvae have never been seen to emerge from any other part of the body indicate that the loss of larvae during the experiment probably took place through the proboscis. This loss was caused either by the overpacking with larvae and subsequent rupturing of the proboscis or by the stimulus exerted by the feeding upon cotton pads soaked with sugar-water solution. These conclusions agree with those arrived at by Galliard (1941).

Although the loss of infective larvae through the proboscis during the week following their attainment of the infective stage took place under experimental conditions, it is probable that the factors bringing about this loss are such that it could be expected to occur under natural conditions.

SUMMARY AND CONCLUSIONS

Culex quinquefasciatus females were allowed to feed at night on a volunteer whose peripheral circulation contained microfilariae of the periodic strain of *Wuchereria*

bancrofti. The engorged mosquitoes were kept in screened cages in which there was at all times cotton soaked with sugar-water solution. Beginning with 16½ days after infection dissections were made every two days through 30½ days, and a few were examined at 39½ days. The numbers of larvae recovered were recorded for the respective regions of the body wherein they were found, i.e., head, proboscis, thorax, and abdomen.

A total of 209 dissections was made. Twenty-six mosquitoes were dissected for each time interval with the exception of the 16½- and 39½-day dissections, which were of 21 and 6 respectively.

During the 14-day interval from 16½ to 30½ days after infection there was a decrease in the average number of larvae per mosquito from 48.7 to 8.8. This loss was not statistically significant until after 18½ days, and the rate of loss had markedly decreased by 26½ days. After this time the average per mosquito remained virtually the same although there was some evidence of a very slight continuing loss. The 6 examined after 39½ days averaged 1.5 larvae per mosquito.

The thorax, which originally contained all the larvae during their development, averaged a lower count on each successive interval until 26½ days, after which time the decrease was not very marked.

The abdomen contained its greatest number in the earlier dissections, decreasing rapidly in count until 22½ days. Subsequently, the counts remained about the same with some minor fluctuations.

The average counts within the head and proboscis varied in a manner similar to one another although the former averaged the higher number of larvae. The averages in these parts of the body increased rapidly after 16½ days and maintained a maximum between 18½ and 20½ days. After this there was a gradual decrease, with a slight rise at 26½ days, until the termination of the experiment. The 6 mosquitoes dissected at 39½ days contained no larvae in either the head or proboscis.

A comparison of the larval counts of the various sites with one another and with the total counts revealed that the larvae, after they had reached the infective stage, left the thorax wherein they had developed and, while a few might have gone forward to the head and proboscis, the majority went to the abdomen first. They apparently stayed there only a short time and then migrated forward to the head and proboscis.

The data indicate that the larvae left the mosquito in considerable numbers after 18½ days of infection. It must be assumed that they made their exit from the proboscis for the following reasons: Larvae were seen to emerge in considerable numbers from the proboscis when the tip of the latter was dipped into saline; some proboscides gave evidence of having been ruptured prior to dissection; larvae were never seen to leave any other part of the body.

After the majority of the larvae had left the mosquito by 24½ days following infection, the remainder seemed to migrate back and forth from abdomen to head with a few leaving the proboscis occasionally. However, the numbers of larvae were so few by that time that conclusions regarding their migration habits cannot be drawn with accuracy.

It was concluded that the larvae escaped from the mosquito either while it was feeding on sugar-water or when the proboscis spontaneously ruptured probably as a result of its being too tightly packed with larvae. Loss of larvae resulting from these causes could be expected during the week following the attainment of the infective stage under natural conditions.

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CERCARIA OREGONENSIS, N. SP., A DERMATITIS-PRODUCING SCHISTOSOME CERCARIA FROM THE PACIFIC NORTHWEST

DOROTHY G. MACFARLANE AND RALPH W. MACY
Reed College

A case of severe dermatitis suffered by a friend after swimming in a local lake suggested the possibility that a schistosome cercaria capable of producing swimmer's itch might be present in this area. The search for such an organism resulted in the discovery of a new species of schistosome cercaria experimentally capable of producing dermatitis in man. A description of the new cercaria and studies on its biology are presented.

Of the local snails examined only *Physa ampullacea* (Gould) was found to be infected. In the years 1943 and 1945, when investigation was undertaken, this species of snail was abundant only in the autumn, and it was then that the infections of *Cercaria oregonensis* were found. Snails were collected and taken to the laboratory where they were isolated in half-pint milk bottles.

The cercaria and other larval stages were studied in the living state unstained or stained with vital dyes, neutral red or nile blue sulphate. In some cases a mixture of the two was employed. If a deep stain was desired, it was obtained by staining the specimens in a watch glass for several minutes. The usual procedure, however, was to pass a drop of the very dilute stain under a cover glass.

The schistosomes were stained and fixed according to the methods of Talbot (1936). The cercariae were placed in a solution of neutral red prepared by adding five drops of a saturated solution of the stain to 250 cc of water. Neutral red not only stained the cercariae, but caused them to retain their tails. A solution of 10% formalin (about 250 cc) was heated to the boiling point and was then added to the stained cercariae. Straight specimens, uniform in size, were obtained in this manner.

Cercaria oregonensis, n. sp.

Specific diagnosis: Length of body 0.315 mm; width 0.69 mm; length of tail stem 0.426 mm, width 0.047 mm; length of furcae 0.222 mm; length of head organ 0.097 mm, width 0.048 mm; diameter of ventral sucker, 0.028 mm; distance from ventral sucker to posterior end of body 0.083 mm. Body, tail stem and furcae uniformly spined. Eyespots present. Five pairs of penetration glands, two anterior and three posterior to the ventral sucker. Large ducts lead from the glands to the head organ, which bears spines of slightly larger size than those found on the body and tail. Digestive system and excretory system not completely traced. The flame cell pattern did not appear to differ from that of the closely related *Cercaria elvae* as described by Talbot. The cercaria is capable of producing a dermatitis in man.

Host: Physa ampullacea (Gould).[1]

Locality: Portland, Multnomah County, Oregon, U. S. A.

Of all the schistosome cercariae described to date, *Cercaria oregonensis* is likely to be confused only with *Cercaria elvae*. That this cercaria differs somewhat from measurements of *Cercaria elvae* as given by Talbot can be seen in the table. The tail of *Cercaria oregonensis* is about 0.025 mm longer than that of *Cercaria elvae*, and there is about the same difference in distance from the ventral sucker to the posterior end of the body. The furcae of *Cercaria elvae* are about 0.032 mm longer

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[1] Determined by Dr. Paul Bartsch, U. S. National Museum.

Comparison of Measurements of Cercaria oregonensis and Cercaria elvae*

	Talbot, 1936 Cercaria elvae Average of 50 preserved specimens	*Cercaria oregonensis Average of 30 preserved specimens*
Length of body	0.307	0.315
Width of body	0.067	0.069
Length of head organ	0.097	0.097
Width of head organ	0.042	0.048
Diameter of ventral sucker	0.034	0.028
Distance from ventral sucker to posterior end of body	0.108	0.083
Length of tail stem	0.401	0.425
Width of tail stem	0.046	0.047
Length of furca	0.254	0.222

* All measurements represent millimeters.

than those of *Cercaria oregonensis*. Other measurements, however, are nearly identical with those made by Talbot.

The structure of the sporocyst has been difficult to determine in this study, particularly since snails infected with the schistosome have been found only late in the fall when the infection was in the final stages. When the snails were dissected, the long slender stringy forms of the sporocyst were easily seen on the surface of the digestive gland. The sporocysts are characteristic of the group—they are so interwoven through the snail's tissues that a complete sporocyst cannot be dissected from the snail. The sporocysts obtained were variable in size, measuring from 1.025 mm to 2.4 mm in length.

Only mature sporocysts were seen, but it can be expected that *Cercaria oregonensis* would have the same development as *Cercaria stagnicolae,* determined by Cort and Olivier (1943). This cycle is reported to consist of mother and daughter sporocysts.

All infections of *Cercaria oregonensis* found in the vicinity of Portland were in *Physa ampullacea* (Gould), whereas Talbot and Cort found *Lymnaea stagnalis appressa* Say and *Lymnaea stagnalis perampla* Walker to be the hosts of *Cercaria elvae.* Of the *Physa* snails collected in this study 3.5% were infected with the cercaria. On San Juan Island, Washington, Miller found 8% *Lymnaea stagnalis* snails collected to be infected with *Cercaria elvae.* No snails of the genus *Lymnaea* were found in the Portland area.

After emerging from the snail, *Cercaria oregonensis* swims directly to the lightest part of the container. In swimming position, the tail is usually placed foremost and the body tends to be horizontal. Upon reaching the light the cercaria may hang motionless in the water with the body part downward and the tail almost horizontal with furcae spread apart. In most cases, however, the cercaria swims directly to the wall of the vessel and becomes attached to it with the ventral sucker. When the body is in this position the tail hangs downward and the furcae are relaxed. The position of *Cercaria elvae,* as described by Cort and Talbot (1936) differs considerably, thus:

> *Cercaria elvae* when attached in which (*vide infra*) both the anterior tip and ventral sucker are attached to the surface and the tail is bent back over the body in form of a hook with the furcae crossed.

Cercaria oregonensis was never observed in this position.

Another peculiarity exhibited by *Cercaria oregonensis* is that its time of emergence from the snails is between 6 PM and midnight in contrast to the time of emergence of *C. elvae* which occurs in the late afternoon rather than in the evening. A specimen of *Physa ampullacea* isolated the day before observation, was changed from one container to another at 6 hour intervals. Filtered pond water from the habitat of the snail was used in the experiment. From 6 PM until midnight approximately six hundred cercariae were shed, but in the period between midnight and 6 AM, only one cercaria was shed. Next morning the container showed numerous cercariae again. Snails collected in the morning did not shed many (if any) cercariae until morning. The naturally acquired case of supposed schistosome dermatitis mentioned previously was contracted while swimming at about 10 PM, and thus coincided with the observed time of emergence of the cercariae.

In the case of dermatitis referred to at the beginning of this paper, the diagnosis by the physician consulted was given as "weed rash," although direct evidence of such etiology was lacking. In development and appearance the dermal inflammation resembled closely "swimmer's itch" so common in the North-central states and Canada, where a dermatitis is produced by the penetration of non-human schistosome cercariae.

Two methods were used to produce experimentally a dermatitis with *Cercaria oregonensis* found in the lake on the Reed College campus. The first attempt, which proved unsuccessful, was the method of immersing a foot or arm into a bucket containing several heavily infected snails. The number of cercariae present in such cases was often very large and the water adhering to the skin contained numerous cercariae. The number of cercariae dealt with in this work was never sufficient to obtain a noticeable dermatitis by this method. However, seven persons submitted to the experiment and accordingly each dipped an elbow into a finger bowl containing *Cercaria oregonensis*. Apparently the cercaria did not penetrate as all results were negative.

Success was obtained by placing on the forearm of each of six persons several drops of water taken from a Syracuse watch glass in which a heavily infected snail had been crushed. Two results were positive and the reactions reported are described as follows: As the water evaporated, the cercaria apparently penetrated the skin. The prickling sensation usually noted for other species was intensely felt by one person. In the other case penetration was accompanied by extreme itching which lasted about three hours. At first the infected spot was red in color and later welts appeared. During the next two days tiny papules developed and the area of dermatitis produced discomfort only when irritated as by the rubbing of the sleeve. In neither case did the infection become pustular. The duration of the dermatitis was variable; in one case it disappeared in a week, in the other it lasted for about a month.

Since the above report was written, McMullen and Beaver (1945) have found that *Cercaria elvae* develops to maturity in mallards, domestic ducklings, and canaries. The species was found to be synonomous with *Cercaria ocellata,* which in turn becomes *Trichobilharzia ocellata* (La Valette). Because of the differences between the biology of the cercaria of this species and *Cercaria oregonensis,* the latter appears distinctive.

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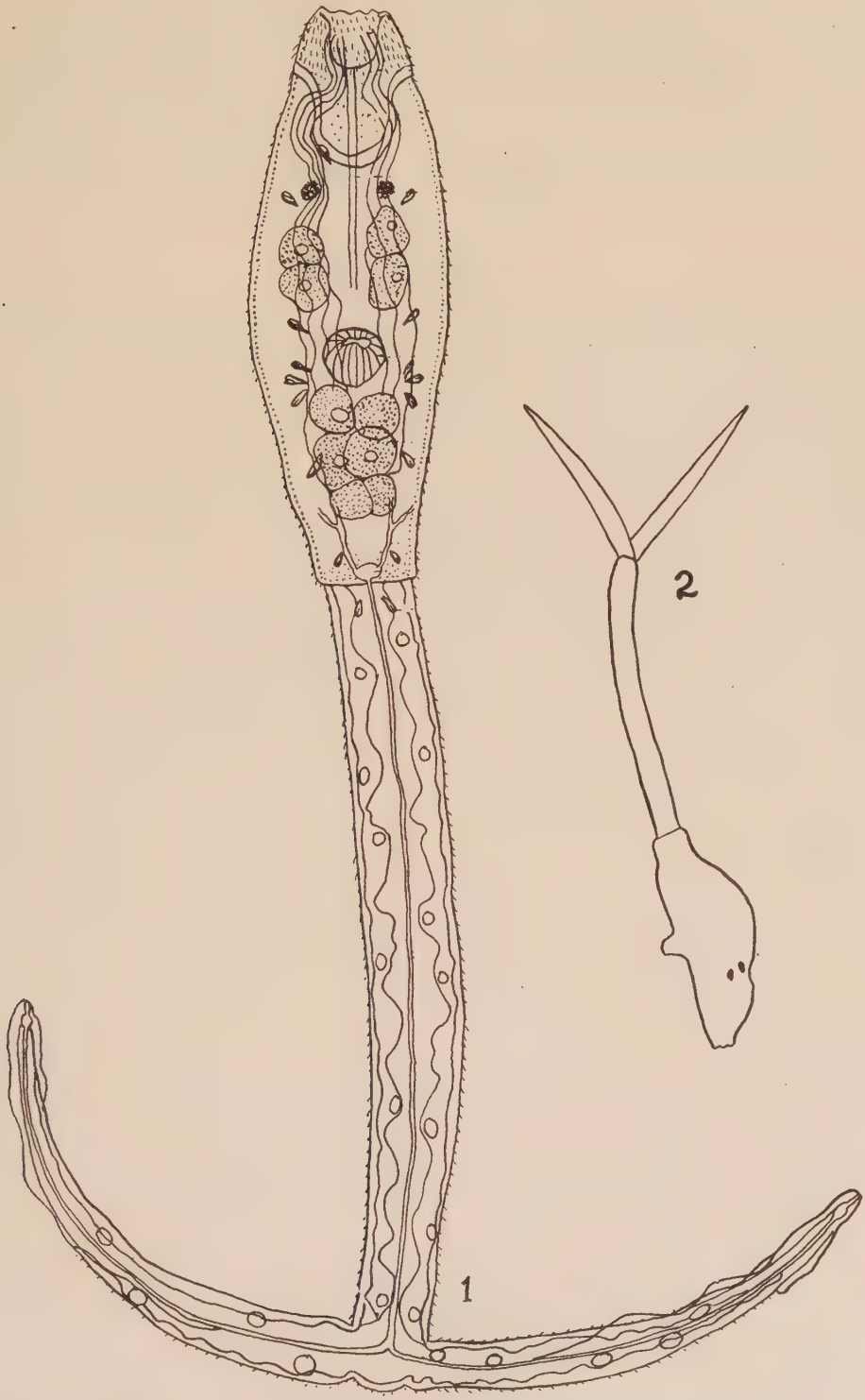

FIG. 1. *Cercaria oregonensis,* n. sp. FIG. 2. *Cercaria oregonensis,* commonly assumed position.

THE GENUS NEOSCHÖNGASTIA (ACARINIDA: TROMBICULIDAE) IN THE WESTERN PACIFIC AREA

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INTRODUCTION

From the winter of 1944 to the fall of 1945 a survey of the trombiculid mites of the Western Pacific Area was conducted by personnel of U. S. Naval Medical Research Unit No. 2 in connection with a program for the elucidation of the epidemiology of scrub typhus or tsutsugamushi fever. During the course of the survey numerous trombiculid mites were collected and studied. The present paper is concerned with the systematics and distribution of the species of *Neoschöngastia* that were known or were found to be present in the area covered by the survey. Little information was available on mites of the genus *Neoschöngastia* found on Pacific islands until the publication of papers by Gunther (1939–1940), by Womersley (1939), and by Womersley and Heaslip (1943).

The authors wish to thank all of the many individuals who participated in the work. Commodore T. M. Rivers MC(S) USNR Medical Officer in Command of U. S. Naval Medical Research Unit No. 2 by his encouragement made the work possible. Lieutenant D. H. Johnson H(S) USNR, Lieutenant (jg) R. H. Baker and the men in their laboratory collected most of the hosts that were examined for larval trombiculids. Many specimens of mites were collected by L. A. Posekany ChPhM, J. M. Fritts PhM1/c, R. K. Carver PhM2/c, J. N. Strong PhM2/c, and C. A. Woodbury PhM3/c. R. E. Muhl PhM3/c gave valuable assistance with the drawings. Thanks are also due to the directors and staffs of the institutions visited and particularly to Dr. H. E. Ewing and Mr. H. Womersley.

Specimens for study were obtained from two sources, museums and the field. The extensive collections of the U. S. National Museum and the South Australian Museum were examined as well as the smaller collections in the School of Public Health and Tropical Medicine, University of Sydney and the Australian Museum. Small collections of ectoparasites were made on New Caledonia, Espiritu Santo, Guadalcanal, Admiralty Islands, and Iwo Jima. Extensive collections were obtained at Empress Augusta Bay on Bougainville Island in the Solomon chain, Guam, Ulithi, Peleliu, Samar, Okinawa and the smaller islands adjacent to them. Trombiculid mites were found at all localities except at New Caledonia and Espiritu Santo. Members of the genus *Neoschöngastia* were found on Bougainville, Iwo Jima, Guam, Ulithi, Peleliu, Samar, and Okinawa.

All of the *Neoschöngastia* taken were found on birds, although numerous trombiculids were collected from other hosts. Many unattached trombiculids were collected at most of the localities where they were found on their hosts, but in no case was a free-living *Neoschöngastia* taken.

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[1] On military leave from Duke University.

[2] The opinions expressed are the authors' and not necessarily those of the Navy Department.

The majority of the specimens were mounted in Gater's solution (30 parts gum arabic, 50 parts water, 50 parts chloral hydrate, and 20 parts glycerin). It was found necessary to bake all slides after they were mounted and then ring them so that the Gater's solution would not pick up moisture from the humid tropical air. In studying the specimens good optical equipment is required. Unless specimens are studied with an oil immersion lens with a numerical aperture of 1.30 or better, many of the important diagnostic features will be overlooked or misinterpreted.

Neoschöngastia Ewing, 1929

Neoschöngastia Ewing, 1929, A manual of external parasites, 187.
Paraschöngastia Womersley, 1939, Trans. Roy. Soc. S. Aust. **63**: 165.
Genotype.—Schöngastia americana Hirst, 1921 Ann. Mag. Nat. Hist. ser. 9, **7**: 37.

Ewing (1929) erected the genus *Neoschöngastia* for those species of *Schöngastia* which lacked a row of dorsal teeth on the chelicerae. He choose as the type species *Schöngastia americana* Hirst, 1921. Womersley (1939) recognized two distinct groups within Ewing's genus *Neoschöngastia*. For that group that had a ridge on the scutum anterior to the pseudostigmata, he erected the genus *Paraschöngastia* and later Womersley and Heaslip (1943) designated *Neoschöngastia yeomansi* Gunther, 1939 as type. Unfortunately *N. americana* and *N. yeomansi* are congeneric. Therefore *Paraschöngastia* is a synonym of *Neoschöngastia*. Ewing who first discovered that *N. americana* and *N. yeomansi* were congeneric agrees with Womersley in that *Neoschöngastia* as originally defined contained two genera. Therefore a new name is required for Womersley's conception of *Neoschöngastia*.

Both Ewing and Womersley did not emphasize what the present authors consider to be the most important characteristic of *Neoschöngastia* s.s. namely, that the scutum or dorsal plate is at least partially submerged beneath the cuticle and therefore the cuticular striations are visible over the surface of the scutum. Three of the new species described here (*N. riversi, N. carveri,* and *N. namrui*) are sufficiently different from other members of the genus, so that on morphological grounds each could conceivably be made the type of a monotypic genus. At present it does not seem advisable to do this because they all fit into *Neoschöngastia* as here defined; they are all parasitic on birds as are the other members of the genus; and additional generic names will confuse rather than clarify the already complex taxonomy of the Trombiculidae.

At least three species of nymphs of *Neoschöngastia* are now known. Two of these nymphs were raised from engorged larvae during the present studies using the techniques described by Wharton and Carver (1946). The third species *Neoschöngastia gallinarum* was described by Kawamura and Yamaguchi (1921).

The nymphs reared in the present study have several characteristics in common: striae over the sensillary area, the same shape of sensillary area, narrowly spatulate pseudostigmatic organs, two types of body setae, one long the other short, on the hysterosoma, no eyes, and six setae on palpal segment four. According to Kawamura and Yamaguchi (1921) the nymph of *N. gallinarum* differs in that the pseudostigmatic organs are broadly spatulate, and but one type of body seta is reported.

On the strength of additional information obtained, a new diagnosis is here given for the genus *Neoschöngastia*. While insufficient knowledge of the nymphal stage of *Neoschöngastia* and related genera makes the diagnosis of the genus on the basis

of nymphal characters conjectural, it is given here as a point of departure for future delimitation of trombiculid genera on nymphal and adult morphology.

Diagnosis.—Larva. With the characters of the family TROMBICULIDAE. Chelicerae with an anteriorly projecting dorsal tooth and recurved ventral tooth so that the apex of the chelicera resembles a conventional can-opener in lateral aspect. Pseudostigmatic organs capitate or strongly clavate. Scutum partially or completely submerged beneath the skin so that the cuticular striae are visible over the surface of at least the posterior part of the scutum. Five scutal setae. Palpal claw variable.

Nymph. With the characters of the family TROMBICULIDAE. (These characters are the same as those previously used to define the genus *Trombicula* in the nymphal or adult stage.) Sensillary area entire, not divided into sections by longitudinal ridges, and reniform with the hilus directed anteriorly. Striae extending over sensillary area. No eyes. Six setae on palpal segment four. Two types of opisthosomal body setae, a long and a short. Pseudostigmatic organs or sensillae spatulate although at times almost filiform.

SPECIFIC DIFFERENTIATION

Among the members of any genus certain morphological features or variations are considered by taxonomists to have "specific" value; others are not. It is realized here that designation of so called "specific" characters is dangerous in that the concept of species is more properly based on genetic rather than morphological grounds. However morphology is the only tool at present available for differentiating species of trombiculids. In identifying larvae, Ewing (1938) has laid great stress on the type of palpal setae, the palpal claw, the chelicerae, and the number and arrangement of the dorsal body setae. Womersley and Heaslip (1943) accentuate the structure and dimensions of the scutum or dorsal plate and have devised a set of standard data (Wharton 1945) for describing it. Gunther (1939), Gater (1932), and others have given more general descriptions of the entire larva including at times the chaetotaxy of the legs. Gunther stressed the difference between setae with barbules on one side or on both sides. Actually the barbules are usually paired and appear to be on only the convex side if the seta is strongly bent or on both sides if the seta is nearly straight. In the present study the following characteristics of the larvae have been carefully considered: setae on the first four segments of the palp, galeal setae, palpal claws, standard data of the scuta, shape and ornamentation of the scuta, scutal setae, sensillae, arrangement and number of dorsal setae, arrangement and number of ventral setae, coxal setae, sensory setae on the tarsi, and eyes. While only two species of nymphs were studied the following characteristics appear to be important: crista metopica, pseudostigmatic organs, palpal thumb, genital suckers, body setae, epistome, and epistomal setae. The dimensions of the segments of leg I that have been used in the past are considered to be of minor importance in comparison with the above characteristics as far as the genus *Neoschöngastia* is concerned.

All dimensions in this paper are given in microns and therefore the word microns is omitted after the measurements.

KEY TO THE SPECIES OF LARVAL *Neoschöngastia* IN THE WESTERN PACIFIC AREA

1. (2) Palpal claw bifurcate, three pairs of sternal setae ..... *N. riversi*
2. (1) Palpal claw trifurcate, sternal setae not as above 3
3. (4) Sternal setae numerous, not paired ..... *N. carveri*
4. (3) Two pairs of sternal setae present 5
5. (6) A single seta on coxa III 7
6. (5) More than one seta on coxa III 19

7. (8) Galeal seta nude 9
8. (7) Galeal seta feathered 17
9. (10) With a hemispherical elevation on the basal segment of the chelicera. *N. bougainvillensis*
10. (9) Without a conspicuous hemispherical elevation on the basal segment of the chelicera 11
11. (12) Dorsal setae fewer than 50 13
12. (11) Dorsal setae more numerous than 50 ..... *N. strongi*
13. (14) Head of sensilla nearly spherical 15
14. (13) Head of sensilla spindle-like with long setules arising from its surface. *N. monticola*
15. (16) Nude seta on tarsus III ..... *N. posekanyi*
16. (15) No nude seta on tarsus III ..... *N. gallinarum*
17. (18) Posterior body setae arising from platelets ..... *N. backhousei*
18. (17) Posterior body setae similar to anterior setae not arising from platelets. *N. egretta*
19. (20) Posterior body setae arising from platelets 21
20. (19) Posterior body setae not arising from platelets 23
21. (22) A girdle of 26 to 30 circular plates at the level of the posterior third of the body. Posterior to the girdle are a number of larger plates each of which bears a single nude seta ..... *N. retrocincta*
22. (21) Posterior body setae all of one type. No girdle or large plates present. *N. yeomansi*
23. (24) More than five setae on coxa III 25
24. (23) Fewer than five setae on coxa III 27
25. (26) Nude galeal seta, PW greater than AW ..... *N. atollensis*
26. (25) Feathered galeal seta, AW greater than PW ..... *N. namrui*
27. (28) Dorsal body setae fewer than 50 ..... *N. americana*
28. (27) Dorsal body setae more numerous than 50 29
29. (30) With as many as three humeral setae on each side ..... *N. pauensis*
30. (29) With a single pair of humeral setae 31
31. (32) Lateral seta on palpal segment four nude ..... *N. dubia*
32. (31) Lateral seta on palpal segment four with a few delicate barbules. *N. ewingi*

Neoschöngastia americana (Hirst, 1921)

Larva, Fig. 1

Schöngastia americana Hirst, 1921, Ann. Mag. Nat. Hist. ser. 9, **7**: 37.
Neoschöngastia americana, Ewing, 1929, A manual of external parasites, 28.

This species was first reported from the domestic chicken from Texas. In the collection of the U. S. National Museum there are specimens from other southern states. The standard data of five specimens follow:

Host	*State*	*AW*	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
Poor-will	Texas	50	67	33	20	23	27	33	50	53	27
Yellow-headed woodpecker	Texas	49	68	26	19	26	26	..	..	..	..
Turkey	S. Carolina	49	62	31	16	29	26	32	55	52	23
Bob-white	Virginia	52	71	34	19	29	31	36	65	65	..
Free-living	Texas	45	65	29	13	24	28	29	49	52	26
Mean		49	67	31	17	26	28	33	55	56	25

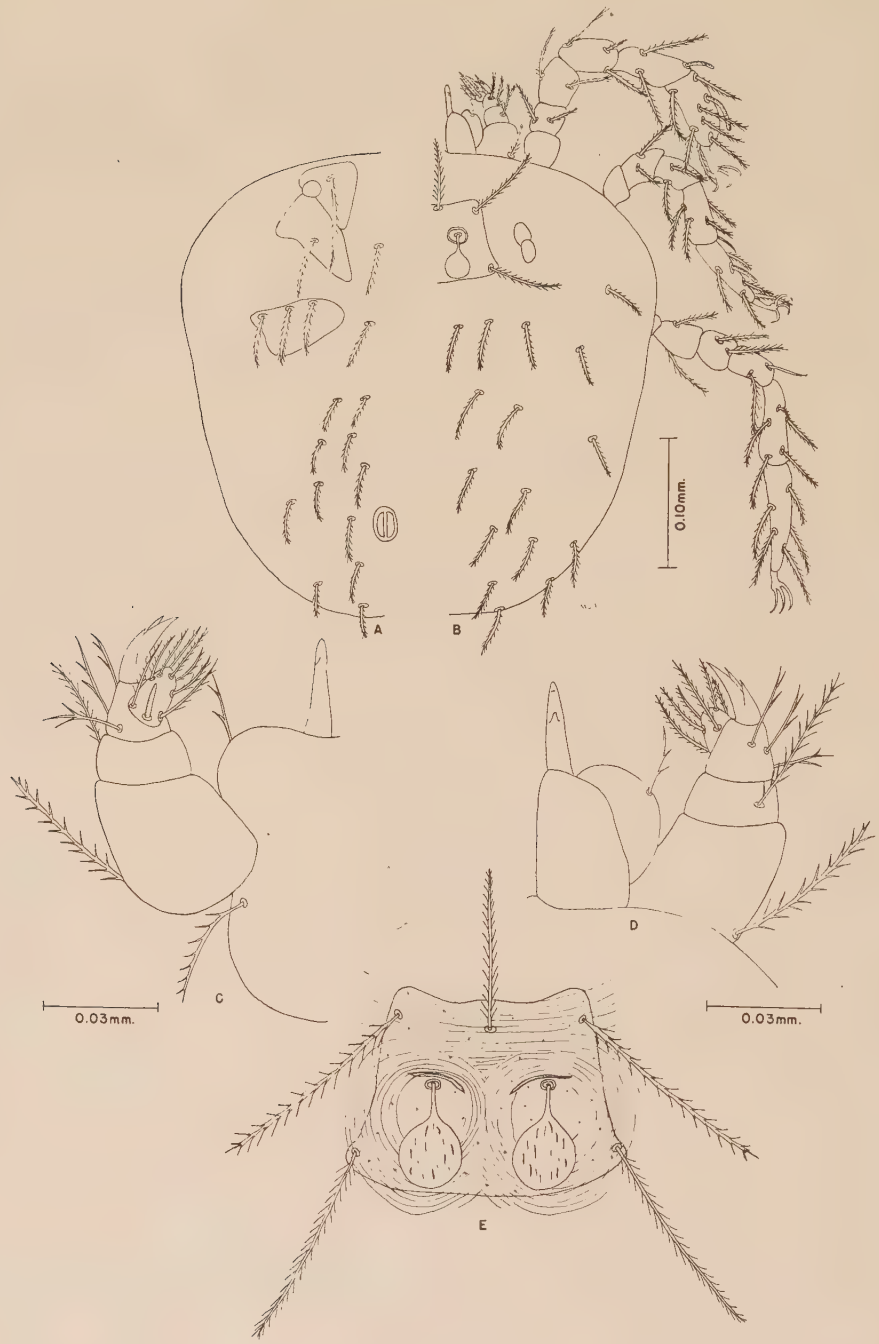

FIG. 1. *Neoschöngastia americana solomonis* n. ssp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

A study of specimens of this species from the Pacific area is given below in detail.

Body: Length 243–500, average of eight specimens 362; width 154–340, average of eight specimens 251. Striations conspicuous over entire body. Color neutral red. Shape oval, but

with broad shoulders. Anterior eye larger than posterior. Body expands posteriorly and dorsally during engorgement. Scutum on anterior face of body in completely engorged specimens.

Gnathosoma: Basal segment of chelicerae long, narrow, and not angular; distal segment curved vertically terminating in a sharp apex; dorsal tooth projects anteriorly, ventral tooth recurved with a median expansion. Palps rounded; segment 1 with a feathered seta; 2 with a feathered seta; 3 with a feathered seta; 4 with 3 setae, dorsal and lateral setae apparently nude but with one or two fine branches, ventral seta obviously feathered, palpal claw trifurcate the middle element the largest; palpal segment 5 with one striated nude sensory seta on the ventral side near the base and seven other setae of which the median dorsal is by far the stoutest. Setae on the cheliceral shield with two to four branches.

Legs: Coxa I and II each with one feathered seta; coxa III with three feathered setae on the anterior border. Striated sensory setae on tarsi I and II. A nude pointed sensory seta on tibiae I and II and genu III.

Scutum: Trapezoidal in shape. Scutal setae with fine short branches on all sides. Entire scutum covered by striated cuticle that masks its pit-like ornamentation of fine stippling. Striae run from right to left in parallel lines anterior to the pseudostigmata and in concentric circles posterior to and around the pseudostigmata which are placed excentrically within the innermost circle. Sensillae or pseudostigmatic organs capitate; the head is approximately spherical and its diameter is about twice the length of the stalk on which it stands and is covered with fine setules that adhere closely to it. In some specimens the head is cordate rather than spherical. The standard measurements of the scutum are:

Bougainville specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	51	67	28	28	28	34	41	53	66	27
	50	71	28	..	..	..	50	61	57	28
Mean	51	69	28	28	28	34	46	57	62	28

Iwo Jima specimen

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
57	77	32	..	..	..	36	44	42	28

Okinawa specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	45	71	39	24	30	35	42	60	60	24
	55	57	35	24	28	34	42	56	59	21
	47	72	35	24	30	38	42	56	56	27
	52	77	38	28	31	36	46	50	56	29
	57	72	39	24	31	35	50	60	64	21
Mean	51	70	37	25	30	36	44	56	59	24

Guam specimen

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
51	68	32	28	28	37	43	60	54	27

Setae: The body setae range in size from 28 to 68. The largest setae are on the anterior dorsum while the shortest ones are posterior and ventral. The most usual formula for the dorsal setae is 2–8–6–4–6–4–2. This formula holds for the majority of specimens from all localities, but some variation is present. The ventral setae are arranged more irregularly than the dorsal. There are two pairs of sternal setae plus 24 to 28 setae posterior to them. There are usually six ventral setae posterior to the anus.

Diagnosis: Neoschöngastia americana can be distinguished from other members of the genus in that it is the only species with three setae on coxae III that has fewer than 50 dorsal setae.

Material: The type and other specimens are deposited in the U. S. National Museum, but representative material will be deposited in the South Australian Museum, Adelaide, South Australia.

Discussion.—The geographical distribution of *N. americana* is typical of many species found in the western Pacific except for its presence in the United States. It

COLLECTIONS OF *Neoschöngastia americana solomonis* N. SSP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
L. A. Posekany	Cape Torokina, Bougainville	*Hirundo tahitica*	3 July 1944	Type + 1
J. M. Fritts	Ypao Pt., Guam	*Anous stolidus*	20 May 1945	1
G. W. Wharton	Iwo Jima	*Monticola solitarius*	29 March 1945	1
L. A. Posekany	Takabannare Shima, Okinawa	*Monticola solitarius*	10 June 1945	10
A. B. Hardcastle	Takabannare Shima, Okinawa	*Monticola solitarius*	10 June 1945	9
M. Markley	Takabannare Shima, Okinawa	Birds	11 June 1945	3
L. A. Posekany	Takabannare Shima, Okinawa	*Monticola solitarius*	17 June 1945	5
L. A. Posekany	Yamanua, Okinawa	*Monticola solitarius*	15 May 1945	1
L. A. Posekany	Hianza Shima, Okinawa	*Monticola solitarius*	25 July 1945	1
A. B. Hardcastle	Hianza Shima, Okinawa	*Streptopelia orientalis*	26 July 1945	2
A. B. Hardcastle	Hianza Shima, Okinawa	*Streptopelia orientalis*	27 July 1945	2
L. A. Posekany	Hianza Shima, Okinawa	*Butorides striatus*	3 Aug. 1945	1

was found most frequently in the Ryukyu Islands near Okinawa on land birds. In the Solomon Islands it was found on only one individual of many hosts examined. The frequency of occurrence of this species on Iwo Jima cannot be estimated since only a single specimen was collected from one of eleven land birds examined on the island. The species is rare on Guam since only a single specimen was found on a noddy and none on the large numbers of land birds examined. The data available indicate that *N. americana* is a parasite of land birds that is distributed widely and frequently by its occasional association with wide-ranging shore-birds. *Anous stolidus* is known to occur on all the land-masses from which this species was taken. At each locality the standard data appear to be distinctive and are indicative of subspeciation. At present it is felt that too little is known about the variation of trombiculid mites to name the specimens from the different islands. However the specimens from the United States and those from the Pacific are different enough to deserve subspecific designation. The specimens from the United States will become *Neoschöngastia americana americana* and those from the Pacific are here designated *Neoschöngastia americana solomonis* n. ssp. after the first locality from which they were taken in the Pacific area. The shorter scutum of *N. americana americana* will distinguish it from *N. americana solomonis*.

Neoschöngastia gallinarum (Kawamura and Yamaguchi, 1921)

Trombicula gallinarum Kawamura and Yamaguchi, 1921, Kitasato Arch. Exp. Med. **4**: 169; Fletcher, Lesslar, and Lewthwaite, 1928, Trans. Roy. Soc. Trop. Med. Hyg. **22**: 161.
Neoschöngastia gallinarum, Sugimoto, 1936, J. Jap. Soc. Vet. Sci. **15**: 201.
Paraschöngastia gallinarum, Womersley and Heaslip, 1943, Trans. Roy. Soc. S. Australia **67**: 130.

N. gallinarum is attributed to Hatori by Kawamura and Yamaguchi, and Sugimoto; however Womersley and Heaslip (1943) failed to find *gallinarum* used as a specific name by Hatori and designated Kawamura and Yamaguchi as the authors of *gallinarum*. Sugimoto (1936) figures what he calls the adult female of *N. gallinarum* but his figure is of a species of *Microtrombidium* as evidenced by the position of the eyes and the presence of accessory palpal claws. Kawamura and Yamaguchi (1921) do figure a nymph of *Neoschöngastia* that they attribute to *N. gallinarum*.

Diagnosis: Larva. No specimens of *N. gallinarum* have been available for examination in the present study. Sugimoto (1936) reports: setae on palp segments 2, 3, and 4 feathered, galeal seta nude, one feathered seta on each coxa; palpal claw trifurcate; dorsal setae 12–8–8–6–8–2 (text) 2–10–8–8–6–2–8–4–2 (figure). Kawamura and Yamaguchi describe the dorsal seta on palpal segment 4 as nude, and report the absence of a nude seta on tarsus III. Womersley and Heaslip (1943) compute the following standard data after Kawamura and Yamaguchi (1921): AW 52—PW 60—SB 32—ASB 16.5—PSB 26.5—AP 31—AM 32—AL 35—PL 50—S 22.5, dorsal setae 2–12–10–8–4–2; and after Fletcher, Lesslar, and Lewthwaite (1928): AW 52—PW 60—SB 42—ASB 19—PSB 26—AP 30—AM 34—AL 35—PL 33—S 26, dorsal setae 2–12–8–6–10–4–2. *N. gallinarum* is the only species of *Neoschöngastia* from the Pacific area whose larva has a trifurcate palpal claw, a nude galeal seta, a single seta on coxa III, two pairs of sternal setae, fewer than 50 dorsal setae, spherical sensillae, and no nude setae on tarsus III.

Nymph. The broadly clavate pseudostigmatic organs of the nymph of *N. gallinarum* will distinguish it from the two other nymphs of *Neoschöngastia* that are described here.

Hosts: Chicken, Fletcher, Lesslar, and Lewthwaite (1928); *Gallus gallus domesticus, Passer montanus tiwanensis, Caprimulgus affinis monticola,* Sugimoto (1936); *Corvus coronoides colonorum, Alcedo atthis japonica, Centropus bengalensis lignator,* Sugimoto (1936) (after Hatori).

Localities: Formosa, Sugimoto (1936); Federated Malay States, Fletcher, Lesslar, and Lewthwaite (1928).

Material: Type and 19 paratypes are in the Taihoku Imperial University Museum, Taihoku, Formosa according to Sugimoto (1936).

Remarks.—As a parasite of the domestic fowl this species may be of economic importance where it is extremely prevalent.

Neoschöngastia yeomansi Gunther, 1939

Neoschöngastia jamesi Gunther, 1938 (nom. nud.), Med. J. Australia **2**: 202.
Neoschöngastia yeomansi Gunther, 1939, Proc. Linn. Soc. New So. Wales **64**: 81.
Paraschöngastia yeomansi, Womersley, 1939, Trans. Roy. Soc. So. Australia **63**: 166; Womersley and Heaslip (1943), Trans. Roy. Soc. So. Australia **67**: 131.

Except for the confusion of Gunther's (1938) *nomen nudum* the synonomy of *N. yeomansi* is uncomplicated.

Diagnosis: N. yeomansi is more nearly like *N. dubia* than any other species of *Neoschöngastia.* The differences existing between the two species are discussed under *N. dubia.* Both of these species are closely related to *N. backhousei,* but differ from it in that the scuta are larger and the lateral setae on palpal segments 4 are nude instead of feathered. *N. yeomansi* was originally described as having a single seta on coxa III; however, later study by Gunther (1940) revealed that in reality two setae could be seen on coxa III. A study of five specimens from Peleliu and of five specimens from New Guinea given to the authors by Womersley, shows that the number of coxal setae may vary from two to four. All of the New Guinea specimens have two setae on coxa III. Of the Peleliu specimens: one has two setae on the left coxa III and three setae on the right; three have three setae on each coxa III and one has four setae on each coxa III. *N. yeomansi* and *N. dubia* therefore also differ from *N. backhousei* in that *N. backhousei* has only a single seta on coxa III. The standard data for *N. yeomansi* are: AW 74—PW 98— SB 63—ASB 26—PSB 25.5—AP 33—AM 38—AL 77—PL 54 (average of nine paratypes, after Womersley and Heaslip 1943); AW 70—PW 93—SB 62—ASB 27—PSB 28—AP 36—AM 38—AL 66—PL 51 (Peleliu specimen). There are feathered hairs on palpal segments 1, 2, 3, and the ventral aspect of 4. The dorsal and lateral hairs of palpal segment 4 are nude. The palpal claw is trifurcate. The galeal seta is feathered. Gunther (1939) in his

original description states that there is a long nude seta on tarsus III. No nude setae are present on tarsus III although there is a long seta with minute branches. The type and paratypes of this species have been examined and except for the differences noted, Gunther's descriptions are complete and accurate.

COLLECTIONS OF *Neoschöngastia yeomansi*

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. N. Strong	Eastern Peninsula, Peleliu Is.	*Gallus gallus*	13 Sept. 1945	2
J. N. Strong	Garakayo Is., Palau Is.	*Megapodius laperouse*	19 Sept. 1945	3

Localities: Bulolo, Territory of New Guinea, Gunther (1939); Lae, Territory of New Guinea, Womersley unpublished record; Peleliu and Garakayo, Palau Islands.

Material: Type and paratypes at the School of Public Health and Tropical Medicine, University of Sydney; paratypes at the Australian Museum, Sydney, New South Wales, and the South Australian Museum, Adelaide, South Australia; Namru No. 2 specimens at the U. S. National Museum, Washington, D. C.

Remarks.—The record of *N. yeomansi* on the Palau Islands from an incubator bird indicates that the species may be limited in distribution to the range of *Megapodius* and its near relatives. Four megapodes from the Solomon Islands were examined; however, no *N. yeomansi* were found. Its occurrence on the domestic chicken is probably rare since it was not found on any chicken examined by Gunther (1939) and only on one of eleven chickens examined in the Palau Islands. The additional numbers of coxal setae on specimens from Palau differentiate them from those from New Guinea, however it is felt that present knowledge of *N. yeomansi* is too fragmentary to establish subspecies.

Neoschöngastia dubia Gunther, 1939

Neoschöngastia incerta Gunther, 1938 (nom. nud.), Med. J. Australia **2**: 202.
Neoschöngastia dubia Gunther, 1939, Proc. Linn. Soc. New So Wales **64**: 89.
Paraschöngastia dubia, Womersley (1939), Trans. Roy. Soc. So. Australia **63**: 165.

As with other species of chiggers described by Gunther (1939) *N. dubia* was identified with a *nomen nudum* of Gunther (1938).

Diagnosis: N. dubia was established for a unique imperfect specimen which has probably been lost. Examination of the Gunther collection in the School of Public Health and Tropical Medicine in Sydney, Australia, failed to locate the type of *N. dubia.* This species differs from *N. yeomansi* mainly in that the scutum is of a slightly different shape. The standard data after Womersley and Heaslip (1943): AW 75— PW 98—SB 60—ASB 25—PSB 25—AP 38—AM 45—AL 80—PL 71 are within the range of *N. yeomansi* except for AP, AM, and PL which are greater by 5, 4, and 13 microns respectively. The greatest difference between the species was thought by Gunther to be the fact that *N. dubia* had three setae on coxa III and *N. yeomansi* one seta on coxa III. In reality *N. yeomansi* may have three setae on coxa III (see above). The hairs on the first four palpal segments like *N. yeomansi* are: 1, 2, 3, and the ventral aspect of 4 feathered, while the dorsal and lateral setae on 4 are nude. Both species have a trifurcate palpal claw and the number and arrangement of the dorsal and ventral setae are similar, however, the posterior dorsal setae are reported not to differ markedly from the anterior ones in *N. dubia* as they do in *N. yeomansi.*

Host: Megapodius duperreyi.

Locality: Bulolo, Territory of New Guinea.

Material: Reported (Gunther, 1939) to be in the School of Public Health and Tropical Medicine, University of Sydney, Australia (see above).

Neoschöngastia backhousei Gunther, 1939

Neoschöngastia fournieri Gunther, 1938 (nom. nud.), Med. J. Australia **2**: 202.
Neoschöngastia backhousei Gunther, 1939, Proc. Linn. Soc. New So. Wales **64**: 89.

Paraschöngastia megapodius Womersley, 1939, Trans. Roy. Soc. So. Australia **63:** 166.
Paraschöngastia backhousei Gunther, 1940, Proc. Linn. Soc. New So. Wales **65:** 252; Womersley and Heaslip (1943), Trans. Roy. Soc. So. Australia **67:** 130.

As with the other names of Gunther (1939) *N. backhousei* is identified with a *nomen nudum*. *N. megapodius* of Womersley (1939) is an error that was pointed out by Womersley and Heaslip (1943).

Diagnosis: N. backhousei is more closely related to *N. yeomansi* and *N. dubia* than to any other species of the genus. It can be separated from them by the characteristics discussed under *N. yeomansi*. The type of *N. backhousei* was examined in the South Australian Museum in order to establish the characteristics of the palps. Palpal segments 1, 2, 3, and the lateral and ventral aspects of 4 each have a single branched or feathered seta. The dorsal seta on palpal segment 4 is nude. The galeal hair is feathered and the palpal claw trifurcate. Gunther (1939) described the lateral hair on palpal segment 4 and the galeal hair as being nude. In other respects his description agrees with that above. Dorsal setae 2–14–14–10–8–8–6–6–2–2; ventral setae 2–2–8–4–6–8–2–2–6–4; a single seta on each coxa III (after Gunther 1939). Standard data: AW 58—PW 72— SB 48—ASB 23.5—PSB 25—AP 42—AM 37.5—AL 47—PL 42—S 30 (after Womersley and Heaslip 1943).

Host: Megapodius duperreyi, Gunther (1939).

Locality: Bulolo, Territory of New Guinea, Gunther 1939.

Material: The type of this species was examined in the South Australian Museum, Adelaide, Australia, where it was on loan from the School of Public Health and Tropical Medicine, University of Sydney, Sydney, Australia.

Neoschöngastia retrocincta Gunther, 1939

Neoschöngastia retrocoronata Gunther, 1938 (nom. nud.), Med. J. Australia **2:** 202.
Neoschöngastia retrocincta Gunther, 1939, Proc. Linn. Soc. New So. Wales **64:** 87.
Paraschöngastia retrocincta, Womersley, 1939, Trans. Roy. Soc. So. Australia **63:** 166; Gunther (1940), Proc. Linn. Soc. New So. Wales **65:** 252; Womersley and Heaslip (1943), Trans. Roy. Soc. So. Australia **67:** 131.

Except for Gunther's original *nomen nudum* there is no confusion in the use of *N. retrocincta*.

Diagnosis: N. retrocincta is unique in that there is a girdle of 26 to 30 circular plates at the level of the posterior third of the body. Each of these small plates bears a stout seta beset with short spines. Posterior to the girdle there are a number of larger plates each of which bears a single nude seta. The chelicerae, scutum, and palps of *N. retrocincta* are typical of the genus. There is a feathered hair on palpal segments 1, 2, 3, and the ventral and lateral aspect of 4; a nude hair is present on the dorsal face of 4. The palpal claw is trifurcate. The galeal seta is feathered. This description of the palpal setae agrees with Gunther (1939) except that Gunther did not see the lateral seta on palpal segment 4, and overlooked the barbs on the galeal seta.

Gunther (1940) re-emphasizes his erroneous description of the galeal seta, "The setae on the cheliceral sheaths (previously described as "apparently nude") are long, slender, straight, and nude." Examination of two of Gunther's paratypes reveals that the setae on the cheliceral shield, galeal setae, are not nude but have three or four fine branches. These setae are difficult to see and the only satisfactory way to examine them is with an oil immersion lens with a numerical aperture of 1.30 or better. Gunther also reports a long nude seta on tarsus III which could not be found by the authors. The dorsal setate are arranged 2–8–12–6–8–8–8 + the setae on the plates described above; the ventral setae 2–2–10–12–10–8–4–6, + the setae on the plates (after Gunther, 1939). The average of the standard data of four specimens including the type and three paratypes are AW 70—PW 85—SB 55.5—ASB 21—PSB 24—AP 35—AM 36—AL 70—PL 48—S 36 (after Womersley and Heaslip 1943).

Hosts: Megapodius duperreyi, Gunther (1939).

Locality: Bulolo, Territory of New Guinea, Gunther (1939).

Material: Gunther (1939) states that he deposited the type specimen in the School of Public Health and Tropical Medicine, University of Sydney, Sydney, Australia. One of the authors (G.W.W.) was unable to locate it there. Womersley and Heaslip (1943) report having measured the type. Paratypes are in the School of Public Health and Tropical Medicine, University of Sydney, Sydney, Australia and the South Australian Museum, Adelaide, South Australia. Identified material is in the U. S. National Museum, Washington, D. C.

Remarks.—Womersley gave one of us (G.W.W.) a slide of *N. retrocincta* that was collected by R. N. McCulloch from a mound in the scrub near Lae, Territory of New Guinea. It is possible that the mound referred to on the label was the nest of the incubator bird *Megapodius duperreyi* the host of *N. retrocincta.*

Neochöngastia bougainvillensis n. sp.
Larva, Fig. 2

Body: Length 306 to 384, width 212 to 256; oval in shape with inconspicuous shoulders. Striae over the entire body strong to moderate. Eyes relatively large, sub-equal. Color reddish orange. All specimens partially engorged.

Gnathosoma: Palpal segment one with a conspicuously feathered seta that originates laterally just posterior to the attachment of palpal segment two. Palpal segment two rounded laterally with a feathered seta on the dorsal surface situated laterally and posteriorly. Palpal segment three with a feathered seta less heavily branched than the setae on palpal segments one and two. Palpal segment four with a nude dorsal seta, an apparently nude lateral seta (a single very fine branch was observed on this seta in one specimen) and a definitely branched ventral seta. Palpal claw trifurcate, the central element the longest and stoutest. Palpal segment five with a single striated sensory seta and at least six feathered setae the dorsal one of which is by far the stoutest. Galeal setae nude. Chelicerae well developed, basal segment unique in that there is a hemispherical elevation on its dorsal surface; distal segment curved dorsally, dorsal tooth projects anteriorly, apex recurved laterally to form a definite apical tooth, ventral tooth minute and recurved.

Legs: Each coxa with a single feathered seta. Nude setae on telofemora II and III; on genua I, II, and III; on tibiae I, II, and III and on tarsus I. Striated sensory setae on tarsi I and II and tibia I.

Scutum: Trapezoidal with expanded corners; anterior margin sinuous, posterior margin indented medially. Ornamentation consists of pitting and concentric striae over the posterior region. Posterior to the pseudostigmata are a pair of pits that can accommodate the sensillae. A ridge guards the pseudostigmata anteriorly and medially. Scutal setae of one type, feathered. Sensillae capitate, head egg-shaped, stalk and head with conspicuous short barbs. The standard data follow:

Bougainville specimens

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
63	92	32	31	25	44	61	61	60	38
55	85	28	28	31	42	56	61	61	34

Guam Specimens

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
57	84	27	30	24	36	60	60	60	34
60	90	30	30	24	45	64	60	60	36

Setae: Dorsal setae feathered 33 to 57 long, the anterior setae are longer; about 90 in number; a single pair of humeral setae, the rest of the dorsal setae arranged in obscure rows of about 10 setae except at the extreme posterior end where the number of setae per row is less. Ventral setae feathered, 10 to 30 long, sternal setae the longest; two pairs of sternal setae plus about 80 posterior setae; about 16 setae posterior to the anus.

Diagnosis: Neoschöngastia bougainvillensis is the only species that has a hemispherical elevation on the basal segment of the chelicera. It can also be distinguished by the fact that it is the only species with the following characteristics: trifurcate palpal claw, nude galeal seta, a single seta on coxa III, more than 50 dorsal setae, and elongated rather than spherical capitate sensillae.

Collections of *Neoschöngastia bougainvillensis* n. sp.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
L. A. Posekany	Cape Torokina, Bougainville	*Hirundo tahitica*	3 July 1944	1
W. Beecher	Cape Torokina, Bougainville	*Hirundo tahitica*	14 July 1944	Type
J. M. Fritts	Ypao Pt., Guam	*Anous stolidus*	24 May 1945	2
C. A. Woodbury	Facpi Pt., Guam	*Heteroscelus incanus*	19 Sept. 1945	1

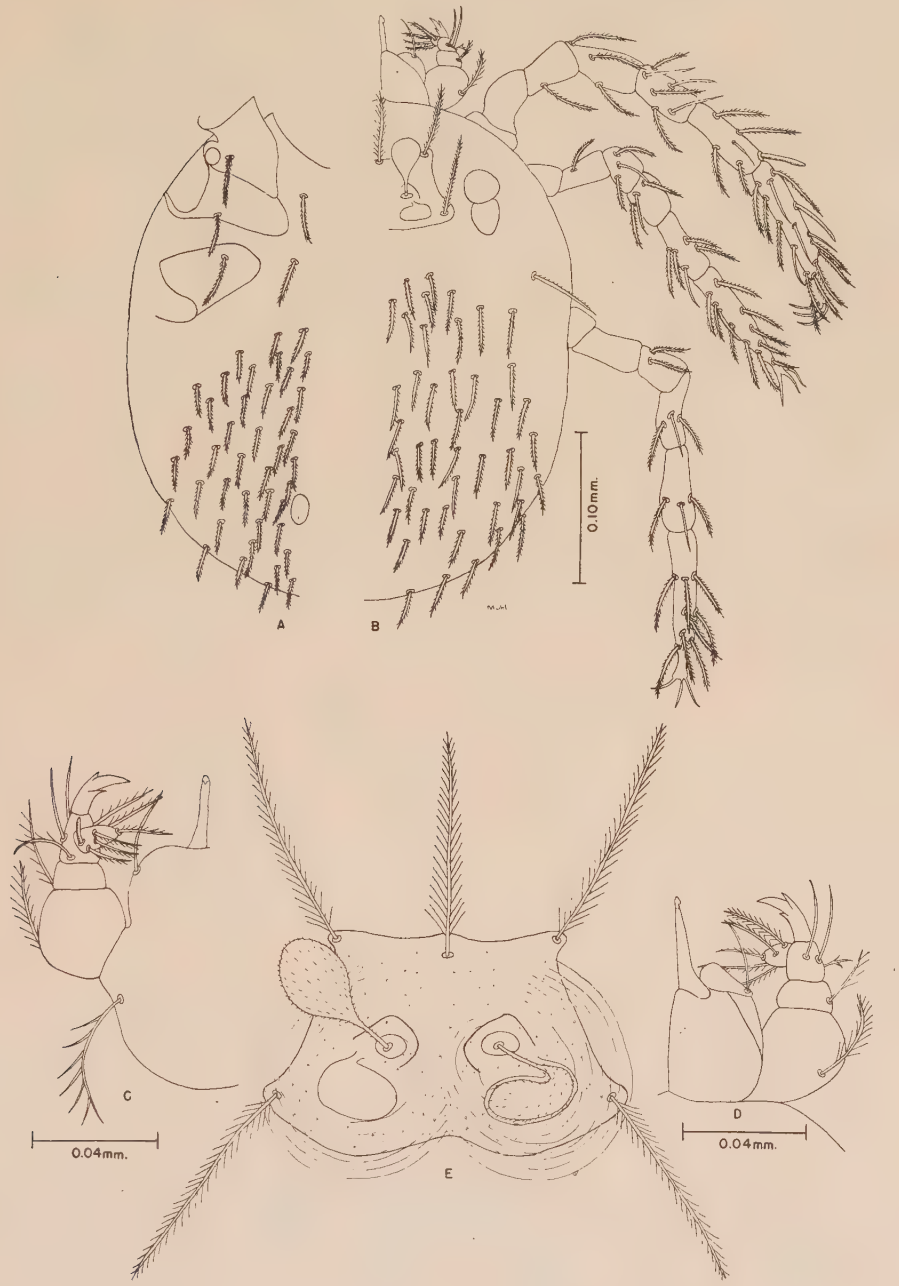

FIG. 2. *Neoschöngastia bougainvillensis* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Material: The type and other material has been deposited in the U. S. National Museum. Identified specimens will be sent to the South Australian Museum, Adelaide, South Australia.

Discussion.—This species is rare on both Bougainville and Guam. Its parasitism on shore birds helps to explain its presence on two widely separated oceanic islands. The species is named for Bougainville Island, the locality from which it was first collected.

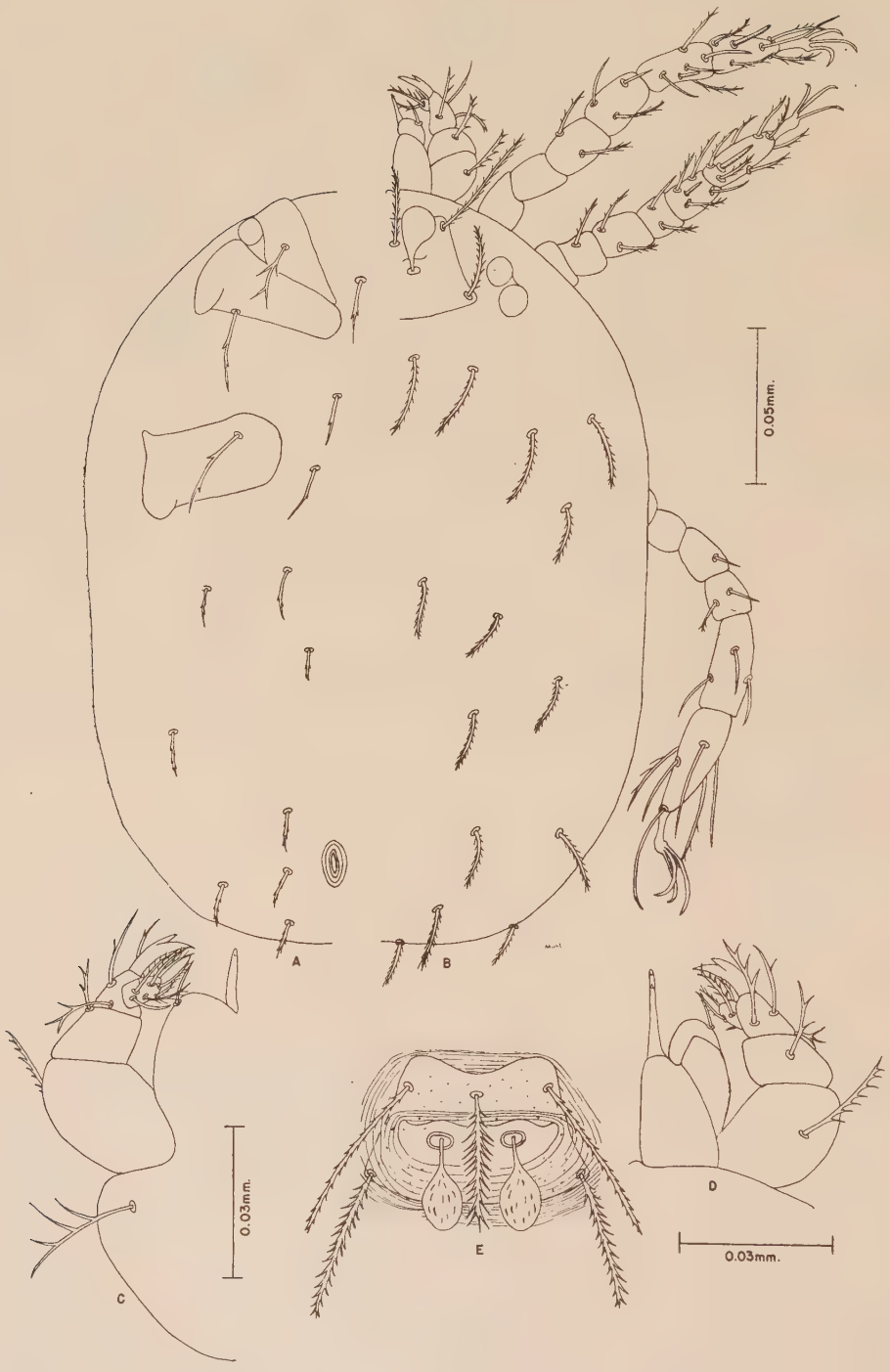

FIG. 3. *Neoschöngastia riversi* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Neoschöngastia riversi n. sp.
Larva, Fig. 3

Body: Length 263–384, average of five specimens 281; width 164–230, average of five specimens 207. Shoulders inconspicuous, shape oval and relatively constant. Striae moderate to weak. Eyes well developed the posterior eye larger, but less conspicuous. Color a light orange.

Gnathosoma: A branched seta originates from the anterior lateral corners of palpal segment 1. Setae on palpal segments 2 and 3 feathered. Dorsal and lateral setae of segment 4 obscurely branched, ventral seta conspicuously so. A heavy branched seta originates from the dorsal lateral surface of segment 5, while five finer branched setae are to be found ventral to it. A striated sensory seta is located on the ventral side of segment 5 near the proximal margin. The paired setae of the cheliceral shield are small and nude. Chelicerae with elongate basal segments. A distal chitinous segment is provided with a forward projecting dorsal tooth, a small broad ventral tooth, and a sharp anteriorly directed apex.

Legs: A single seta with two or three branches on each coxa. Tarsi I and II each with a striated sensory seta. Tarsus three with nude whip-like setae. Tibiae I and II each with nude setae and a small striated seta. Additional nude setae on genua I and II, and telofemur, genu, and tibia III.

Scutum: Trapezoidal in shape. The anterior margin is concave, while the posterior margin is convex. The anterior-lateral setae are situated some distance from the anterior margin. All of the scutal setae are provided with numerous barbs, those on the anterior-lateral setae shortest. The pseudostigmatic organs or sensillae are capitate, the head is egg-shaped and is supported by a thin neck about half the length of the head. The scutum is ornamented by scattered punctae. There is a ridge anterior to the pseudostigmata. The posterior portion of the scutum is imbedded in the skin and the cuticular striations extend over the scutum posteriorly except in the area adjacent to the pseudostigmata. The standard data follow:

Bougainville specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	35	56	17	21	18	21	35	50	40	24
	38	57	17	17	21	24	35	43	40	28
	40	59	18	25	18	28	39	49	40	25
	41	60	21	23	14	23	43	54	45	24
	34	52	17	22	18	24	38	55	49	23
Mean	38	57	18	22	18	24	38	50	43	25

Samar specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	34	58	20	21	21	27	42	50	44	30
	40	57	16	24	20	26	31	60	43	29
	40	57	18	23	18	24	36	58	46	26
	38	57	20	23	15	26	43	60	49	31
	40	60	21	23	18	26	36	58	46	28
Mean	38	58	21	23	18	26	38	57	46	29

Setae: The dorsal setae are similar to the scutal setae. The anterior setae are longer (29) than the posterior setae (21). The dorsal formula in the type is 2–8–6–2–4–4–2. In some specimens the four most posterior setae are on the ventral surface. The ventral setae are smaller than the dorsal setae. They are arranged 2–2–2–4–4–2–4–2. The anus is opposite the penultimate row of ventral setae. The three pairs of sternal setae sometimes appear nude.

Diagnosis: Neoschöngastia riversi is unique in possessing three pairs of sternal setae and in having a bifurcate palpal claw.

COLLECTIONS OF *Neoschöngastia riversi* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. M. Fritts	Cape Torokina, Bougainville	*Eurystomus orientalis*	27 July 1944	Type + 34
L. A. Posekany	Cape Torokina, Bougainville	*Haliaeetus sanfordi*	12 July 1944	6

J. M. Fritts	Cape Torokina, Bougainville	*Falco severus*	18 Oct. 1944	9
J. M. Fritts	Guiuan, Samar, Philippine Is.	*Halcyon chloris*	1 March 1945	1
J. M. Fritts	Guiuan, Samar, Philippine Is.	*Eurystomus orientalis*	11 March 1945	9

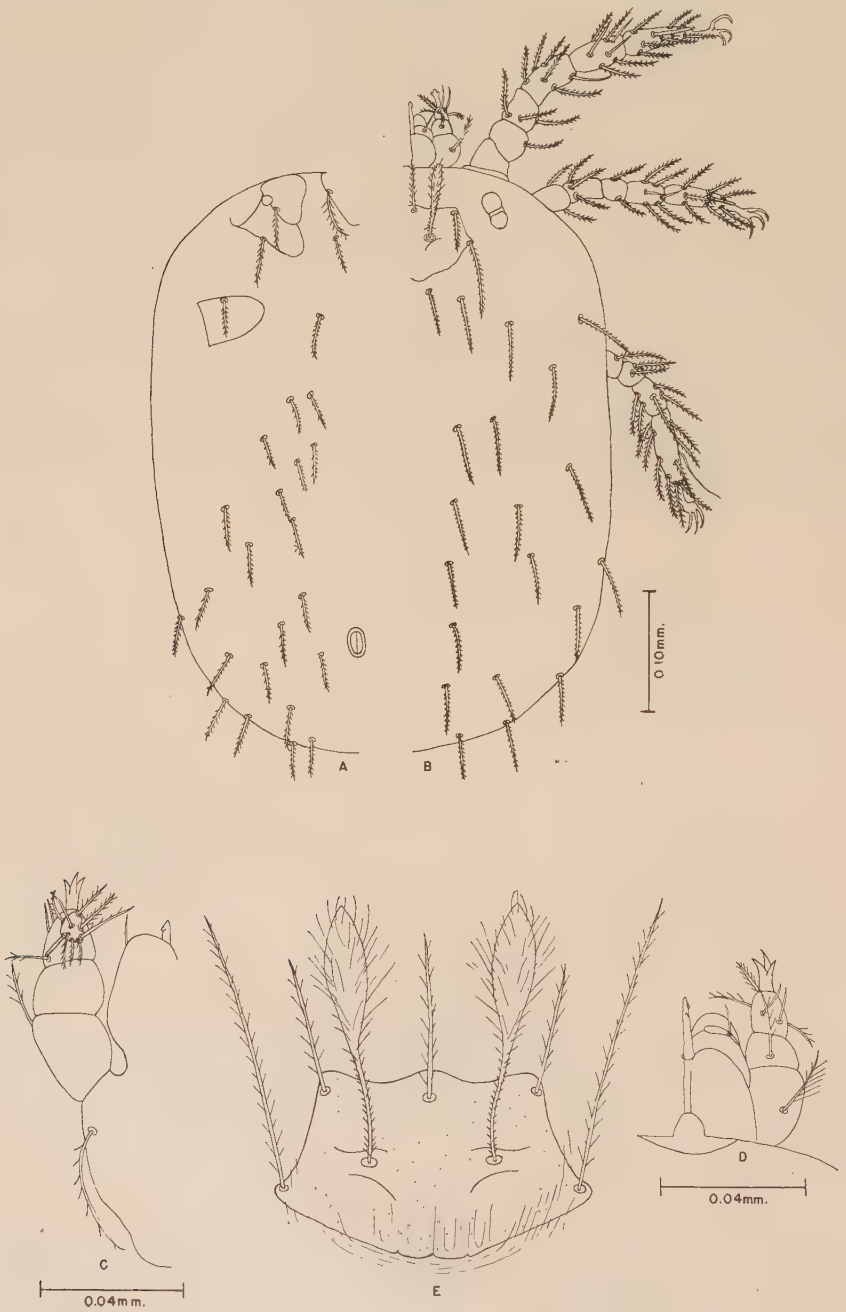

FIG. 4. *Neoschöngastia monticola* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Material: The type and additional identified specimens are in the U. S. National Museum. Identified material will also be sent to the South Australian Museum and the Philippine Academy of Science, Manila, Philippine Islands.

Discussion.—This species is named in honor of Commodore T. M. Rivers MC(S) USNR under whose command this work was done. Specimens from Bougainville and Samar are so similar that they show no evidence of subspeciation.

Neoschöngastia monticola n. sp.
Larva, Fig. 4

Body: Length 282 to 563, width 192 to 492, oval with distinct shoulders. Striae weak. Color yellow. Eyes close together, the anterior pair the larger. A unique plate-like thickening at the anterior margin, the median expansion of which projects between the basal segments of the chelicerae like an epistome. It is 22 wide and 10 long including the projection.

Gnathosoma: Feathered seta on palpal segment one; palpal segment two elongated and rounded with a heavily feathered seta on the dorsal side; palpal segment three with a feathered seta on its dorsal surface; palpal segment four with nude dorsal and lateral setae, and feathered ventral seta; palpal claw trifurcate, the central element longest and stoutest; palpal segment five with one striated sensory seta at the base, one nude seta near the apex, and seven feathered setae of which the dorsal one is by far the stoutest. Galeal hair nude. Basal segment of chelicera stout; distal segment elongated, teeth minute, dorsal tooth projects anteriorly, apex flattened and blade-like, ventral tooth recurved and expanded laterally.

Legs: All coxae with a single feathered seta. A striated sensory seta on tarsi I and II. Nude setae on tibiae I and II and genu I. A nude whip-like seta on tarsus III.

Scutum: Roughly pentagonal with sinuous anterior and posterior borders. Setae provided with numerous small thorn-like branches, setae similar except for length. Ornamentation consists of small pits scattered over the scutum and parallel striae that run in an anterior-posterior direction over the posterior third of the scutum. A pair of ridges one anterior and one posterior guard the pseudostigma on each side. The pseudostigmatic organs are clavate with an attenuated oval head that bears many long setules. The stalk is beset with numerous barbs. The standard data are:

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	60	82	34	27	31	28	47	37	82	70
	54	72	33	24	27	27	49	33	78	75
	54	78	32	24	24	27	36	36	72	63
	54	82	33	24	25	27	36	33	78	75
	57	75	30	24	25	27	45	36	66	
Mean	56	78	32	24	26	27	43	35	75	71

Setae: The dorsal setae are similar to those on the scutum; the larger anterior setae are about 62 long while the posterior setae are about 39. The intermediate setae vary progressively in length between these extremes. The setae are arranged in rows as follows: 2–8–6–6–6–6–4–2, 2–8–6–6–4–6–4–2 or 2–8–6–6–4–4–6–4. In one specimen nine setae were present in the row posterior to the humeral setae. The difference in the arrangement of the dorsal setae is more apparent than real since the relative position of the various setae can change greatly during the engorgement of an individual. The ventral setae are shorter than the dorsal setae and are provided with slightly longer barbs. There are two pairs of sternal setae followed by from 40 to 50 posterior setae arranged in irregular rows. About fourteen ventral setae are posterior to the anus.

COLLECTIONS OF *Neoschöngastia monticola* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
L. A. Posekany	Takabannare Shima, Okinawa	*Monticola solitarius*	10 June 1945	Type + 5
A. B. Hardcastle	Takabannare Shima, Okinawa	*Monticola solitarius*	10 June 1945	19
M. Markley	Takabannare Shima, Okinawa	Birds	11 June 1945	3

L. A. Posekany	Takabannare Shima, Okinawa	*Monticola solitarius*	16 June 1945	8
L. A. Posekany	Takabannare Shima, Okinawa	*Monticola solitarius*	17 June 1945	5
L. A. Posekany	Hianza Shima, Okinawa	*Monticola solitarius*	25 July 1945	3
A. B. Hardcastle	Tsuwa Village, Okinawa	*Monticola solitarius*	9 Aug. 1945	1

Diagnosis: N. monticola is different from all other species of the genus from the Pacific area in that it possesses the epistome-like plate described above. The scutum is also unique in that the posterior margin is scalloped and the striae on the scutum are parallel and run vertically rather than concentrically or laterally.

Material: Type and identified material are in the U. S. National Museum. Identified material will be deposited in the South Australian Museum, Adelaide, South Australia.

Discussion.—N. monticola is apparently confined to the rock thrush. That it was found on this host and only at a locality, in the vicinity of Okinawa, where a large number of rock thrushes were examined indicates that its free-living stages are in some way dependent on the rock thrush's nesting or roosting habits. This species is probably restricted to areas inhabited by the rock thrush or its close relatives. Other species of the genus are restricted to hosts of similar habits. For example *N. retrocincta, N. yeomansi,* and *N. backhousei* are evidently closely associated with incubator birds although *N. yeomansi* was collected once from a wild jungle fowl *Gallus gallus.* Except for nesting habits, however, chickens and incubator birds are similar in behavior. In fact so much so that in New Guinea incubator birds are commonly referred to as jungle chickens.

Neoschöngastia posekanyi n. sp.
Larva, Fig. 5

Body: Length 312 to 412, width 212 to 300, oval in outline with inconspicuous shoulders. Striae moderate. Color orange. Eyes on a level with the anterior lateral setae. The anterior pair are larger.

Gnathosoma: Palpal segment one with a feathered seta medial to the articulation of palpal segments one and two. Palpal segment one more limited posteriorly than in other species. Palpal segment two narrower proximally than distally, shaped like a truncate cone with base situated distally. A feathered seta originates from the dorsal surface of palpal segment two midway between the distal and proximal ends. Palpal segment three has a feathered seta on its dorsal surface. However, the number of branches on this seta is considerably less than on the seta of palpal segment two. Palpal segment four carries the following setae; nude dorsal, feathered lateral, and feathered ventral. The palpal claw is trifurcate. The central element is stout while the two lateral elements are small. The setae on the cheliceral shield, galeal setae, are nude. The basal segments of the chelicerae are relatively large and stout, the distal segments of the chelicerae are short, stout, and strongly curved dorsoventrally. Dorsal tooth projects anteriorly, apex knife-like, ventral tooth is recurved and projects laterally.

Legs: All coxae with a single feathered seta. The setae on coxae II and III longer than on I. A striated sensory seta on tarsi I and II, a long whip-like seta on tarsus III. Nude setae on genua I, II, and III, and on tibiae I, II, and III and tarsus I.

Scutum: Approximately rectangular in shape. Setae of two types, the anterior median seta with a heavy shaft and barbs, the other setae slender with short barbs. Sensillae capitate, head spherical to cordate covered with fine setules. Ornamentation consists of scattered pits anteriorly and horizontal parallel striae posteriorly. Anterior and posterior margins of scutum sinuous. An eyebrow-like ridge anterior to each pseudostigma. The standard data for the scutum follow:

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	78	80	52	22	34	28	50	77	56	28
	77	84	49	27	35	31	49	70	49	27
	74	80	45	27	35	31	49	91	56	29
	77	77	45	27	29	28	52	70	52	35
	70	78	46	24	35	29	45	66	49	
Mean	75	80	47	25	34	29	49	73	52	30

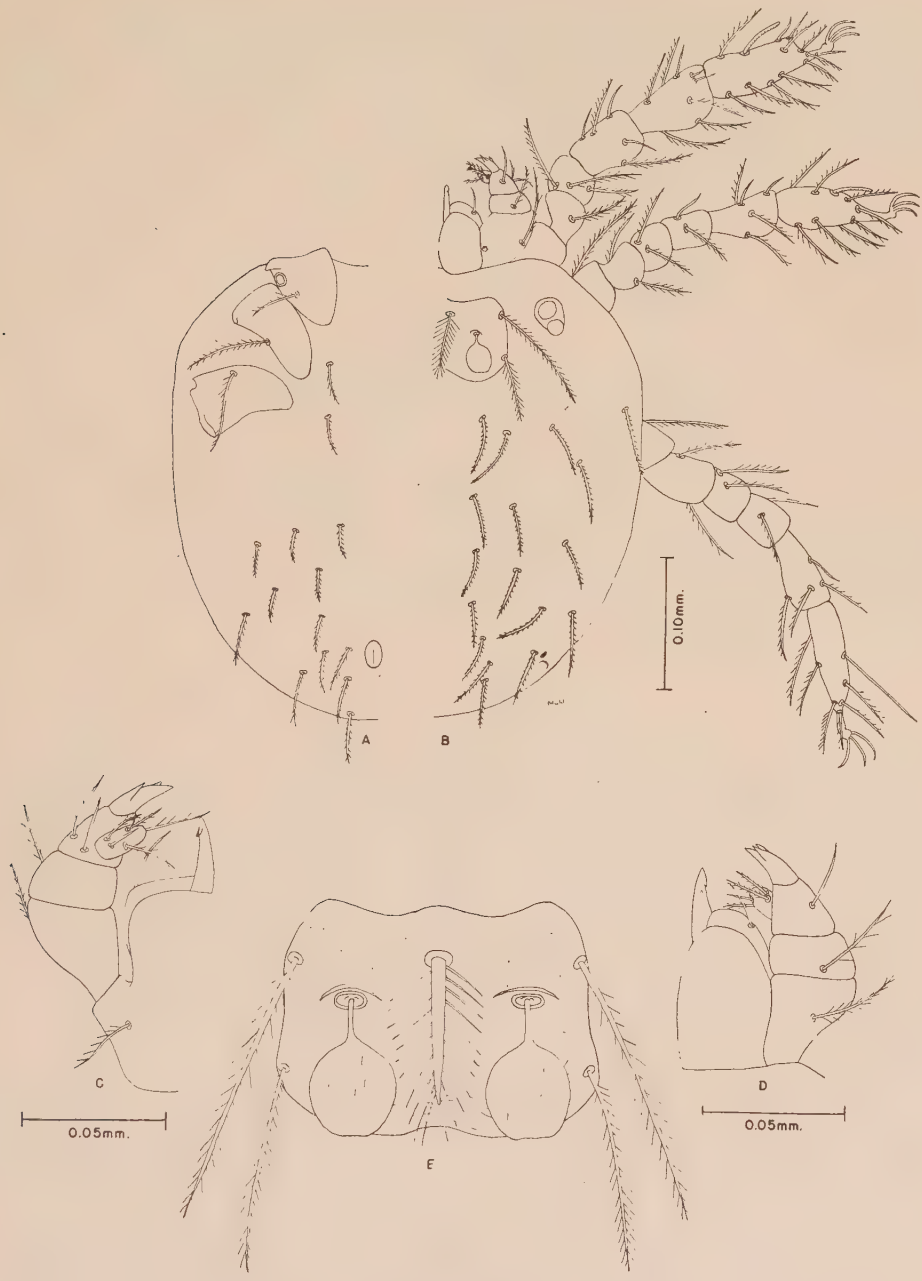

FIG. 5. *Neoschöngastia posekanyi* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Setae: Dorsal setae provided with short barbs. Anterior dorsal setae 52 long, posterior setae 43 long, arranged as follows: 2–8–6–8–4–2–2 2. Some variation is apparent in the arrangement. The first four rows are usually 2–8–6–8 although one specimen had only seven setae in the second row. The posterior rows may be as above or 4–2–2 or 2–4–2. The ventral setae are shorter than the dorsal and arranged in rows approximately as follows: 2–2–6–6–2–6–2–2. Much variation is encountered in the arrangement of the ventral setae, there are at least four ventral setae posterior to the anus. The first two rows of ventral setae are the sternals.

Diagnosis: N. posekanyi is closely related to *N. gallinarum* but differs from it, in that the number of dorsal setae is distinctly less. The presence of a nude seta on tarsus III of *N. posekanyi* separates it from *N. gallinarum*. The striations on the scutum are also of a different nature. In *N. gallinarum* the striae are concentric while in *N. posekanyi* they are horizontal parallel lines. *N. posekanyi* can be distinguished from other species by the following combination of characters: coxa III with a single seta, palpal claw trifurcate, nude galeal seta, dorsal setae less than 50 in number, and capitate sensillae with spherical head.

Collections of *Neoschöngastia posekanyi* n. sp.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
L. A. Posekany	Yamanua, Okinawa	*Monticola solitarius*	15 May 1945	1
L. A. Posekany	Awa, Okinawa	*Monticola solitarius*	21 May 1945	13
A. B. Hardcastle	Hianza Shima, Okinawa	*Streptopelia orientalis*	26 July 1945	2
L. A. Posekany	Hianza Shima, Okinawa	*Streptopelia orientalis*	26 July 1945	Type

Material: Type and other material are placed in the U. S. National Museum. Identified material will be sent to the South Australian Museum, Adelaide, South Australia.

Discussion.—This species was found only on landbirds and only on Okinawa. Like the previous species it probably is limited in range to the range of its normal hosts. It is named after the collector of the first specimens.

Neoschöngastia egretta n. sp.
Larva, Fig. 6

Body: Oval in shape with inconspicuous shoulders, length 279 to 437, width 177 to 280. Striae strong. Eyes opposite sensillae, anterior eye much larger than posterior.

Gnathosoma: Palpal segment one with a feathered seta situated laterally posterior to the articulation of segments one and two. Palpal segment two rounded with a feathered seta placed laterally on the dorsal surface. Palpal segment three with a feathered seta bearing only two or three barbs. Nude dorsal and lateral setae on palpal segment four. Ventral setae of four long, with numerous barbs. Palpal claw trifurcate, the central element the stoutest. Palpal segment five with a striated sensory seta near its base and seven feathered setae of which the dorsal one is the largest. Galeal hair strongly feathered with short barbs. Chelicerae with stout basal segments; distal segment long, curved only at its articulation with the basal segment. Dorsal tooth projects anteriorly, apex pointed, ventral tooth recurved and expanded laterally.

Legs: A single feathered seta on each coxa. Striated sensory setae on tarsi I and II, a whip-like seta on tarsus III. At least four nude setae on tibia III, a nude seta also on genu I and genu III.

Scutum: Roughly rectangular with sinuous anterior and posterior margins. Setae feathered, thickly beset with moderately long barbs, all of the same type. Sensillae capitate with spherical head beset with minute setules. Pitting extends over entire scutum. Striations on the posterior half of the scutum arranged concentrically about pits posterior to the pseudostigmata that will accommodate the sensillae. Ridges anterior to the pseudostigmata. The standard data follow:

Okinawa specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	67	74	50	22	28	29	25	56	40	31
	67	74	55	20	29	31	..	60	35	..
	..	73	55	24	36	35	31	..	35	..
Mean	67	74	53	22	31	32	28	58	37	31

Ulithi specimen

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
62	72	52	21	29	31	27	56	38	..

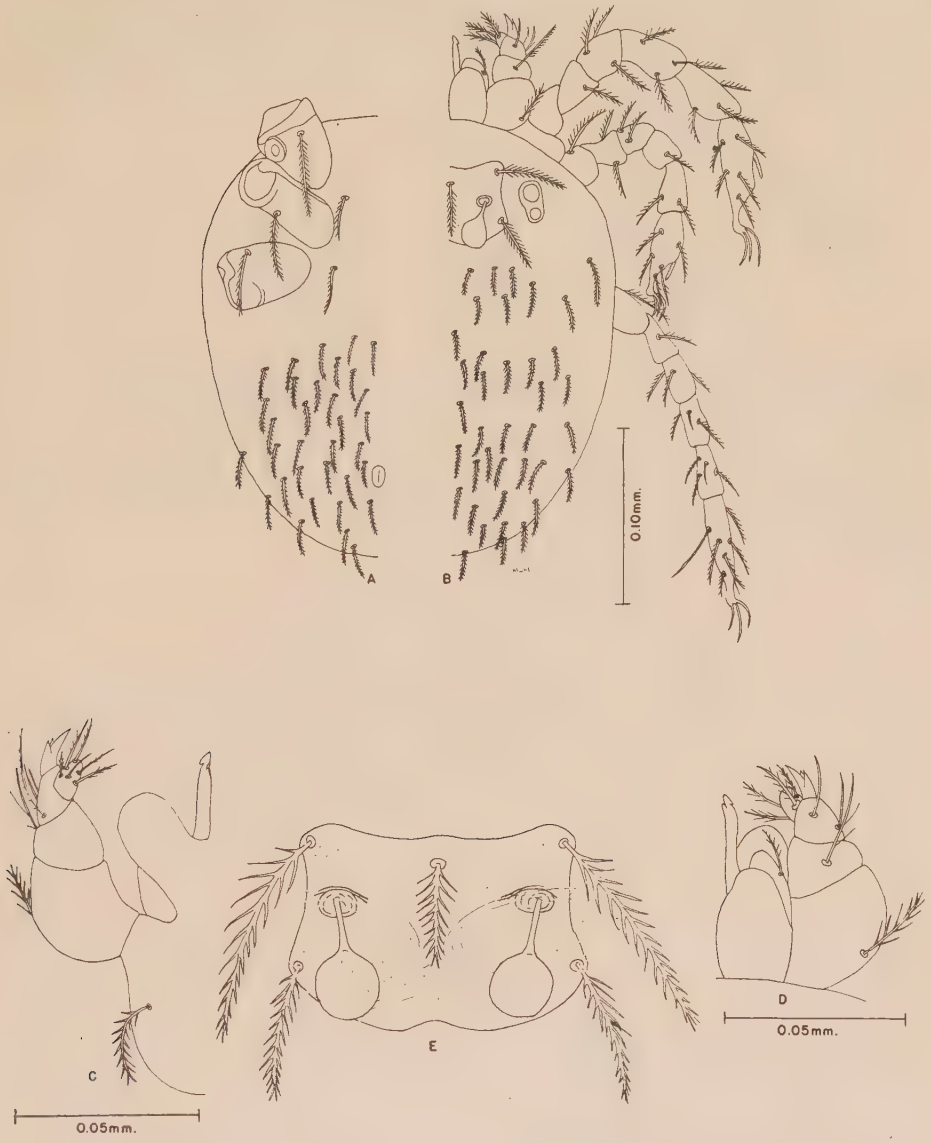

FIG. 6. *Neoschöngastia egretta* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Setae: The dorsal setae are feathered and range from 45 in length at the anterior end to 21 in length at the posterior end. The setae are not arranged in definite rows. There are two humeral setae followed by from 66 to 74 setae which are roughly in rows of ten except at the posterior end. The ventral setae are similar to the dorsal setae. The two pairs of sternal setae are longer than the 64 to 70 ventral setae that are arranged in irregular rows of about 10 posterior to coxae III. The anus is situated in the midline at the level of the posterior third of the group of ventral setae.

Diagnosis: Neoschöngastia egretta differs from *N. backhousei* in that the cuticle at the posterior end is striated rather than pitted and the galeal hair is conspicuously feathered; and from other species in that it possesses the following combination of characters: coxa III with one seta, palpal claw trifurcate, galeal seta feathered, and striae arranged concentrically about the scutal pits posterior to the pseudostigmata.

COLLECTIONS OF *Neoschöngastia egretta* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
L. A. Posekany	Hianza Shima, Okinawa	*Egretta intermedia*	2 August 1945	Type + 2
J. N. Strong	Potangeros Is., Ulithi Atoll	*Demigretta sacra*	15 August 1945	2
J. N. Strong	Potangeros Is., Ulithi Atoll	*Egretta intermedia*	15 August 1945	1
J. N. Strong	Bulubul Is., Ulithi Atoll	*Anous stolidus*	21 August 1945	1
J. N. Strong	Pau Is., Ulithi Atoll	*Pluvialis dominica*	21 August 1945	1

Material: Type and other material are deposited in the U. S. National Museum. Identified specimens will be sent to the South Australian Museum, Adelaide, South Australia.

Discussion.—N. egretta is a rare species on Okinawa and Ulithi, the only two localities from which it was collected. Its presence on shore birds seems to explain its presence on two widely separated oceanic island groups. No evidence of incipient speciation is apparent in the few specimens collected. This species is named for one of its common hosts.

Neoschöngastia atollensis n. sp.

Larva, Fig. 7

Body: An elongated oval with distinct shoulders, length 342 to 400, width 200 to 262. Striae moderate to strong. Eyes large, opposite scutum, anterior eye larger than posterior eye.

Gnathosoma: A feathered seta on palpal segment one. Palpal segment two rounded with a dorsal feathered seta situated posteriorly and laterally. A dorsal feathered seta with two to four barbs on palpal segment three. Palpal segment four bears a dorsal nude seta, a lateral nude seta, and a ventral feathered seta. Palpal claw trifurcate, central element stouter and longer than lateral elements. Palpal segment five with a single striated sensory seta at the base and at least 4 feathered setae. Nude galeal seta. Basal segment of chelicerae stout; distal segment strongly curved with a sharp apex, a small anteriorly projecting dorsal tooth, and a laterally expanded recurved ventral tooth.

Legs: A long feathered seta on coxa I, a short feathered seta on coxa II, and from 5 to 7 feathered setae along the anterior border of coxa III. The number of setae on coxa III is not necessarily the same on the right and left coxae of an individual. Striated sensory setae on tarsi I and II. Nude setae on the genua of all legs, and on tibiae I and II. No whip-like seta on tarsus III.

Scutum: Narrower anteriorly than posteriorly. Posterior lateral margins an even curve. Lateral, anterior, and posterior margins sinuous. Anterior scutal setae much closer to anterior margin of scutum than in other species. Scutal setae feathered with long barbs. Sensillae capitate with spherical head, small for the size of the scutum. Pitting evenly distributed over anterior margin of plate, striae arranged concentrically around depressions which accommodate the sensillae posterior to the pseudostigmata. The pseudostigmata are covered by a sigmoid ridge that is free of ornamentation laterally and whose posterior margin is elevated from the scutum. The standard data follow:

Ulithi specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	46	70	21	21	35	48	25	49	34	..
	47	73	24	20	34	43	27	50	35	..
	48	77	24	21	32	43	28	53	35	32
	48	69	18	18	31	39	28	49	34	..
	48	70	22	22	28	43	28	49	32	..
Mean	47	71	22	20	32	43	27	50	34	32

Okinawa specimen

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
42	56	14	15	27	34	21	36	28	24

Setae: Dorsal setae feathered 49 to 21 long. About four humeral setae on each shoulder. Dorsum with about 120 setae arranged in indistinct rows of 12 or less. Ventral setae also numerous, about 100 posterior to the two pairs of sternal setae. The anus is situated in the center of the group of ventral setae posterior to the coxae.

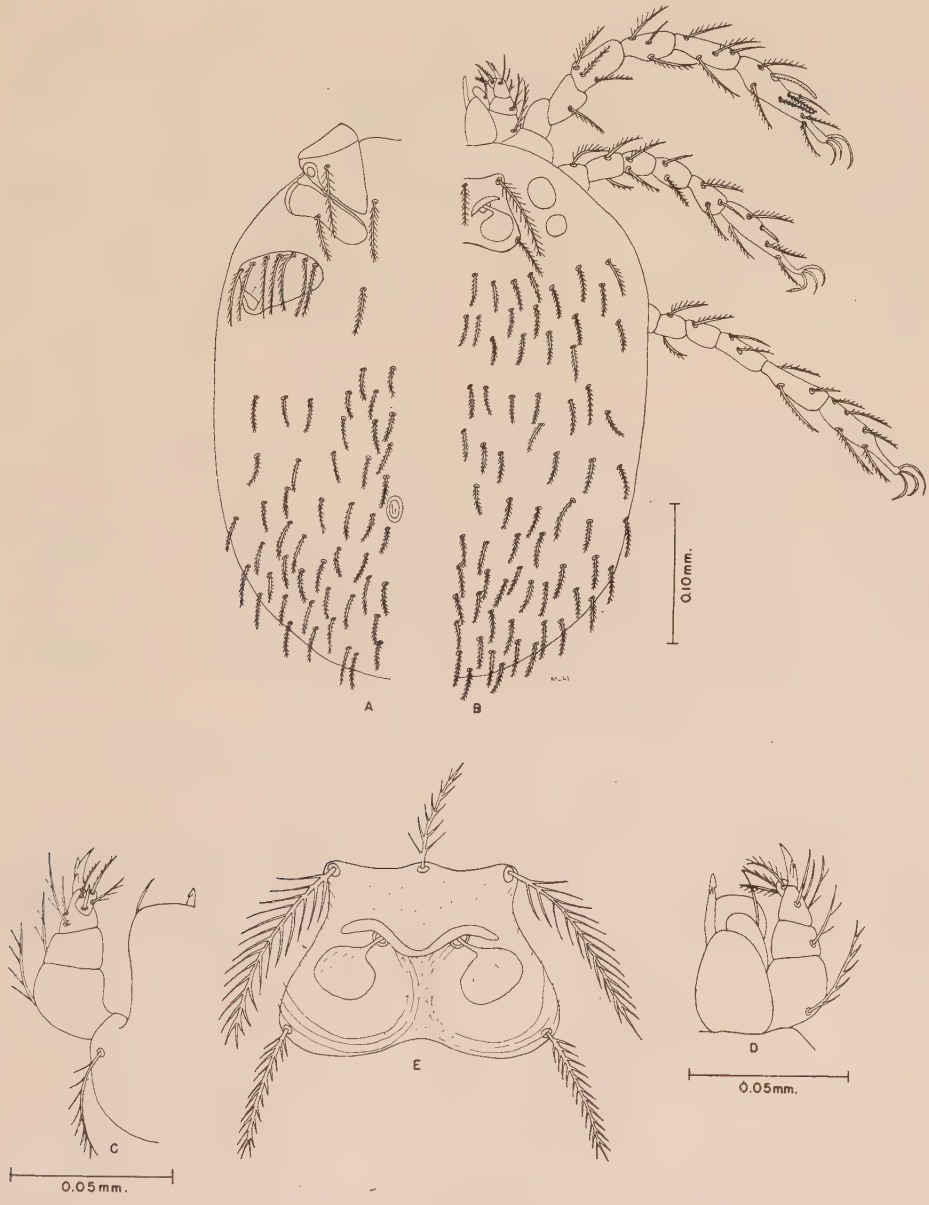

FIG. 7. *Neoschöngastia atollensis* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Diagnosis: Neoschöngastia atollensis can be distinguished from *N. namrui* in that the PW is greater than the AW in the former while in the latter the reverse is true. Many other differences also exist. *N. atollensis* can be differentiated from the other species described from the Pacific area since it exhibits the following characteristics: coxa III with more than 4 setae, and only two pairs of sternal setae.

COLLECTIONS OF *Neoschöngastia atollensis* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
L. A. Posekany	Hama Heka Shima, Okinawa	Birds	30 July 1945	1
J. N. Strong	Potangeros Is., Ulithi Atoll	*Numenius phaeopus*	17 August 1945	Type + 4

Material: Type and other material have been deposited in the U. S. National Museum. Identified material will be sent to the South Australian Museum, Adelaide, South Australia.

Discussion.—The single specimen from Okinawa has a smaller scutum than the specimens collected from Ulithi. However, until additional material is available or until more is known about subspeciation of trombiculid mites it seems unwise to designate it as a different subspecies even though it was collected so far from the type locality. The presence of this species on shore birds helps explain its wide distribution.

Neoschöngastia ewingi n. sp.
Larva, Figs. 8 & 9

Body: Oval with indistinct shoulders. Length 270 to 487, width 155 to 390. Color orange. Striae moderate to strong. Eyes opposite sensillae, anterior eye larger than posterior.

Gnathosoma: A feathered seta on palpal segment one. Palpal segment two elongate with a feathered seta on the dorsal surface. A feathered seta with fewer barbs than on two on palpal segment three. A nude seta on the dorsal surface of palpal segment three, lateral and ventral setae of palpal segment three feathered. There are two or three fine barbs on the lateral seta which are difficult to see. The ventral seta is long and has 6 to 8 barbs. Palpal claw trifurcate, central element the longest and stoutest. Two striated sensory setae on palpal segment five, both on the ventral surface, one near the apex, the other in the usual position at the base. Seven feathered setae on palpal segment five, the dorsal one of which is the stoutest. Galeal hair with one or two barbs. Basal segment of chelicerae elongate. Distal segment curved and provided with conspicuous teeth. Apex sharp and needle-like, dorsal tooth projects anteriorly, ventral tooth large, recurved and expanded laterally.

Legs: Coxae I and II each with a single feathered seta. Coxae III usually with three feathered setae but one specimen has three setae on the left coxa III and four setae on the right coxa III: Tarsi I and II each have a single striated sensory seta. Tarsus III with no nude setae. Nude setae on genua and tibiae I, II, and III.

Scutum: Approximately rectangular in shape. Pitting anteriorly, striae arranged concentrically about the depressions posterior to the pseudostigmata. Anterior margin sinuous, posterior margin sinuous in unengorged specimens but an even curve in engorged specimens. Scutal setae feathered with numerous barbs. Sensillae capitate with spherical head beset with minute setules. A pair of ridges protect the pseudostigmata anteriorly. The standard data are:

Engorged specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	69	80	55	24	29	34	34	59	38	27
	64	85	55	24	27	34	35	59	42	..
	62	84	51	25	28	35	31	55	39	..
	67	80	55	24	32	34	..	62	42	31
Mean	65	82	54	24	29	34	33	59	40	29

Unengorged specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	57	70	49	20	29	32	..	55	42	..
	59	76	52	18	28	32	29	56	43	..
	63	73	45	22	30	32	29	50	42	..
	60	72	46	21	31	34	31	..	38	..
	63	72	50	20	29	32	28	62	42	27
Mean	60	73	48	20	29	32	29	56	41	27

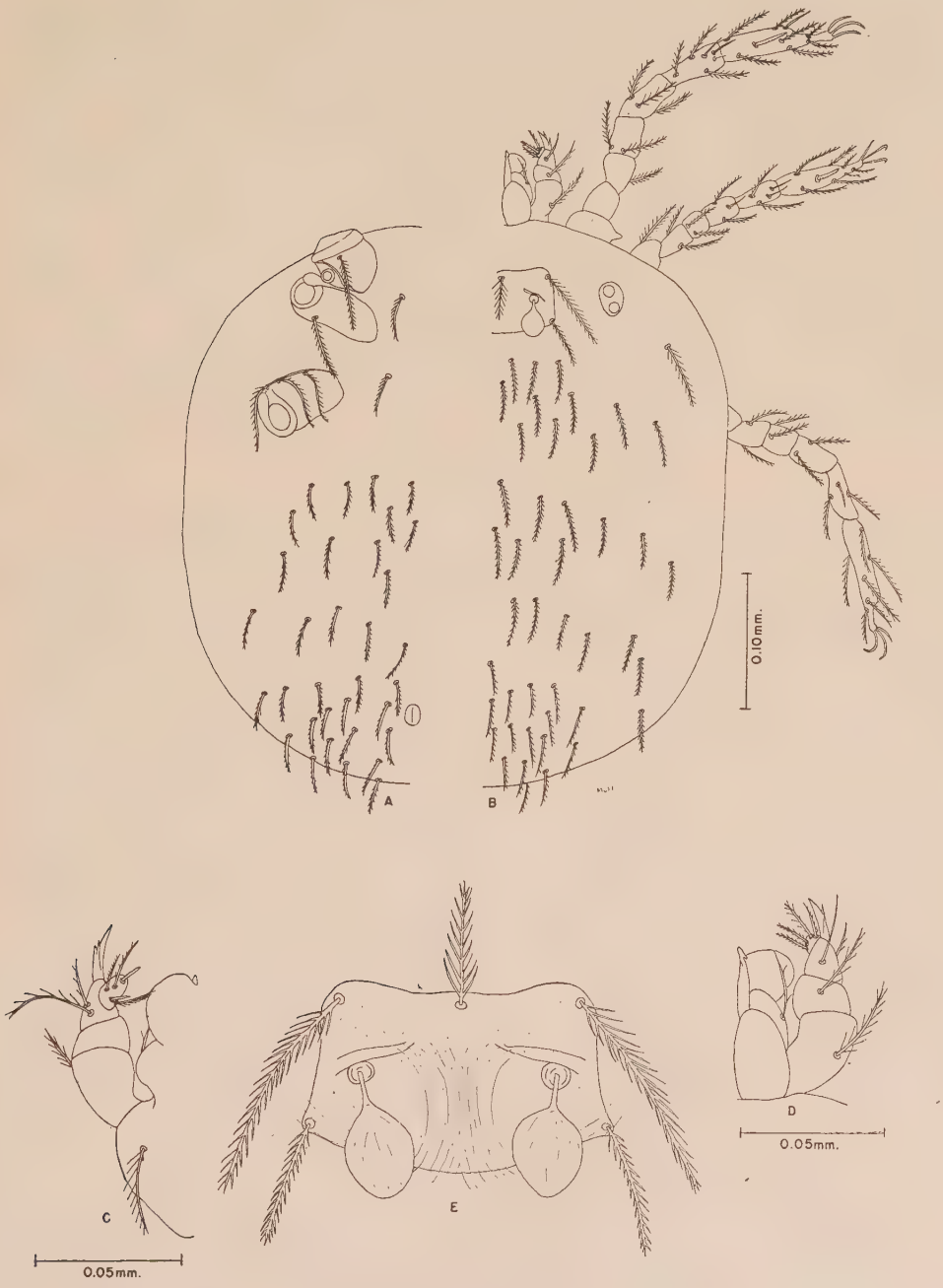

FIG. 8. *Neoschöngastia ewingi* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Setae: Dorsal setae 50 to 21 long and provided with numerous short barbs. Anterior setae longer than posterior setae. A single pair of humeral setae followed by from 60 to 80 or more that are arranged in two poorly defined areas. An anterior group of about 40 to 50 and a posterior one of about 30. Rows indistinct in both groups. Ventral setae shorter than dorsal setae. Two pairs of sternal setae plus about 70 posterior setae. About 16 ventral setae posterior to the anus.

Diagnosis: This species is close to *Neoschöngastia dubia* but differs from it in that there

are two striated sensory setae on each palpal segment five, in place of one. The scutum is smaller in *Neoschöngastia ewingi* and there are no nude setae on tarsus III in *N. ewingi* while three are reported from *N. dubia.* The following combination of characters will separate *N. ewingi* from other species in the Pacific area: coxa III with 3 or 4 setae, PW not much greater than AW, number of dorsal setae greater than 50, and posterior dorsal setae not on platelets.

FIG. 9. *Neoschöngastia ewingi* n. sp. Scutum of unengorged specimen. ×500.

COLLECTIONS OF *Neoschöngastia ewingi* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. N. Strong	Potangeros Is., Ulithi Atoll	*Egretta intermedia*	15 Aug. 1945	2
J. N. Strong	Potangeros Is., Ulithi Atoll	*Pluvialis dominica*	16 Aug. 1945	3
J. N. Strong	Fassari Is., Ulithi Atoll	*Gallus gallus*	19 Aug. 1945	8
J. N. Strong	Pau Is., Ulithi Atoll	*Pluvialis dominica*	21 Aug. 1945	2
J. N. Strong	Bulubul Is., Ulithi Atoll	*Pluvialis dominica*	21 Aug. 1945	3
J. N. Strong	Ngajangel Is., Palau Is.	*Gallus gallus*	2 Sept. 1945	3
J. N. Strong	Ngabad Is., Palau Is.	*Gallus gallus*	11 Sept. 1945	Type + 2
J. N. Strong	Eastern Peninsula, Peleliu Is.	*Gallus gallus*	13 Sept. 1945	1
J. N. Strong	Garakayo Is., Palau Is.	*Gallus gallus*	19 Sept. 1945	3
J. N. Strong	Anguar Is., Palau Is.	*Gallus gallus*	21 Sept. 1945	3
C. A. Woodbury	Facpi Pt., Guam	*Heteroscelus incanus*	28 Sept. 1945	3

Material: Type and other material have been deposited in the U. S. National Museum and identified material will be deposited in the South Australian Museum, Adelaide, South Australia.

Discussion.—During the study of specimens of this species it was at first thought that two species were represented. Specimens were separated into two groups on the characters of the scutum. Specimens in which the striae extended in to the depression posterior to the pseudostigmata and in which the posterior margin was sinuous were placed in one group (Fig. 9). Specimens which had the striae arranged differently and with the posterior margin an even curve were placed in a second group (Fig. 8, E). No other significant differences were found except

measurements of the scutum. All of the specimens of one group were found on chickens while those of the other were found on chickens and shore-birds. In searching for further differences, it was noted that the size of one group was smaller than that of the other. The smaller specimens had a sinuous posterior margin on the scutum and the PW was also considerably smaller. When a chigger engorges it expands posteriorly and laterally. Lateral expansion of the scutum would result in a straightening of the scutum and an increase in the measurement of PW. The striae of the scutum are not in the substance of the scutum itself but are the striae of the body cuticle. In *Neoschöngastia* the posterior end and at times the entire scutum is beneath the cuticle. In the present case as the cuticle was pulled posteriorly by the expanding opisthosoma the striae over the scutum receded and came to lie in the position in which they were found in the second group. In this case it is clear that the character of the scutum can change with engorgement of the individual. Too much emphasis must not be placed on the standard data, if mistakes are to be avoided.

Neoschöngastia pauensis n. sp.

Larva, Fig. 10

Body: Oval with fairly conspicuous shoulders. Length 487 to 512, width 300 to 362. Striae strong. Eyes opposite the sensillae, anterior eye larger than the posterior one.

Gnathosoma: A feathered seta on palpal segment one just posterior to the articulation of palpal segments one and two. Palpal segment two rounded and elongate, dorsal seta feathered and situated on the posterior lateral margin. Seta on third palpal segment feathered but with fewer barbs than on the seta of palpal segment two. Dorsal and lateral setae of palpal segment four nude, ventral setae long and feathered. Palpal claw trifurcate, the central element the stoutest and longest. Galeal seta with two barbs. Palpal segment five has two striated sensory setae and seven feathered setae of which the dorsal one is the largest. Basal segment of chelicerae stout, distal segment relatively small. Dorsal tooth projects anteriorly, apex blade-like, ventral tooth larger than dorsal and expanded laterally.

Legs: Coxae I and II with a single feathered seta. Coxa III with three or four feathered setae along its anterior margin. Of the four specimens collected, three have four setae on coxa III and one has three setae on coxa III. A striated sensory seta on tarsi I and II. No whip-like seta on tarsus III. Nude setae on genua I, II, and III, tibiae I and II, and tarsus I.

Scutum: Lateral margins concave, anterior and posterior margins sinuous. Scattered pits over entire scutum, except in regions around the anterior median, median seta and the pseudostigmata. Striae over the posterior two-thirds arranged concentrically around the pseudostigmata which are excentric in relationn to the striae being situated along the anterior limit of the concentric rings formed by the striae. Scutal setae feathered with numerous barbs. The barbs on the anterior median seta are longest, those on the anterior lateral setae are intermediate, while the posterior lateral setae bear the shortest barbs. Sensillae capitate with a spherical head that is beset with small setules. An eyebrow-like ridge immediately anterior to each pseudostigma. The standard data follow:

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	59	77	38	..	31	32	38	56	53	31
	56	77	40	22	27	32	31	49	56	..
	59	77	35	..	..	29	36	..	56	28
	56	77	36	22	34	32	35	..	56	29
Mean	56	77	37	22	31	31	35	53	55	29

Setae: The dorsal setae are feathered and from 55 long at the anterior end to 30 at the posterior end. There are as many as five humeral setae on each shoulder. The posterior setae are about 50 in number and arranged in indistinct rows of about 10 setae anteriorly and fewer setae posteriorly. The ventral are about the same length as the dorsal setae. There are two pairs of sternal setae and about 60 setae behind the coxae. About twenty of the ventral setae are posterior to the anus.

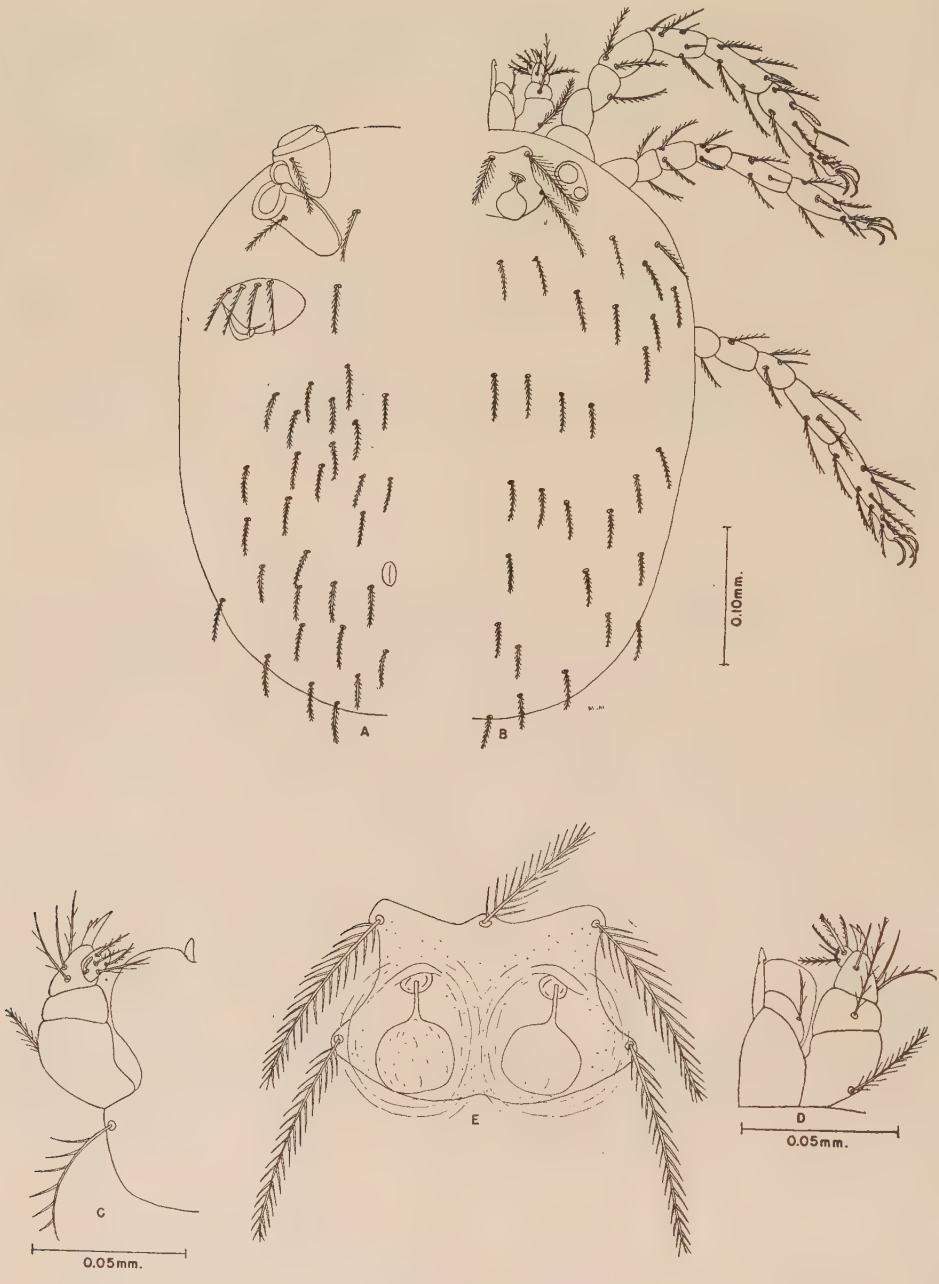

FIG. 10. *Neoschöngastia pauensis* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Diagnosis: Neoschöngastia pauensis is close to *N. ewingi* but can be distinguished from it readily by the presence of numerous humeral setae on *N. pauensis,* rather than a pair as on the shoulders of *N. ewingi. N. ewingi* and *N. pauensis* are the only species that have two striated sensory setae on palpal segment 5 and they are similar in that the scuta are much alike as are the setae on the legs. *N. pauensis* differs from other species in that it possesses the following characteristics: coxa III three to four setae, posterior setae do not arise from platelets, more than one pair of humeral setae.

COLLECTIONS OF *Neoschöngastia pauensis* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. N. Strong	Pau Is., Ulithi Atoll	*Pluvialis dominica*	21 August 1945	3
J. N. Strong	Bulubul Is., Ulithi Atoll	*dominica Pluvialis*	21 August 1945	Type

Material: Type and other material will be deposited in the U. S. National Museum.

Discussion.—A rare species in the area studied. Probably not a native of Ulithi Atoll but carried from a more northern locality by the southward migrating shore birds on which it was found.

Neoschöngastia carveri n. sp.

Larva, Fig. 11

Body: Oval with indistinct shoulders. Length 362 to 650, width 220 to 463. Color orange red. Striae moderate. Eyes large, especially the anterior eye which is more than twice the diameter of the posterior eye. Ocular plate opposite scutum and almost as large.

Gnathosoma: Feathered seta on palpal segment one lateral and just posterior to the articulation of palpal segments one and two. Palpal segment two rounded with a dorsal feathered seta at the posterior lateral margin. Palpal segment three with a feathered seta that has fewer barbs than the seta on palpal segment one. Dorsal and lateral setae of palpal segment four nude, ventral setae feathered. Palpal claw trifurcate, the middle element the stoutest. Fifth palpal segment with one striated sensory seta, one nude seta at the tip, and seven feathered setae of which the dorsal is by far the stoutest. Galeal seta conspicuously feathered with four to six barbs. Basal segment of chelicera long, distal segment with blade-like apex, dorsal tooth blunt, projects anteriorly, ventral tooth recurved smaller than dorsal tooth.

Legs: Coxae I and II each with a feathered seta with numerous short barbs. Coxa III with from six to eight feathered setae along the anterior margin. Of five specimens only one has the same number of setae on the right and left coxae III. An extremely long striated sensory seta on tarsus I, a shorter striated sensory seta on tarsus II. No whip-like setae on tarsus III. Nude setae on tibiae and genua of all legs. Genu I exceptional in that there are at least eight short nude setae on its dorsal aspect.

Scutum: Roughly rectangular but with a sinuous anterior border and a strongly concave posterior margin. Ornamented with scattered pitting over the entire surface except in the region of the anterior median seta. Striae on the posterior third which radiate from the concavity in the posterior margin. Anterior median seta stout with numerous long barbs. Anterior lateral setae narrow with short barbs, posterior lateral setae attenuated with barbs intermediate in length. Sensillae capitate with spherical heads beset with fine setules, stalks extremely narrow at base. Parallel ridges in front and behind the pseudostigmata. The standard data are:

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	81	87	57	33	36	48	69	75	162	39
	72	78	36	29	36	42	75	72	108	36
	81	81	40	28	36	46	69	72	144	42
	75	80	39	30	36	42	60	66	135	36
	90	93	45	30	39	48	81	84	136	48
Mean	80	84	43	30	37	45	71	74	141	40

Setae: All body setae feathered, dorsal setae 93 to 66 long, the anterior setae the longest. Humeral setae arranged in patches of from eight to ten on each shoulder, posterior and medial to these are about one hundred setae. Ventral setae shorter than dorsal setae. Sternal setae not in pairs but in irregular rows, usually about twenty-two sternal setae. About one hundred setae posterior to the sternals, about twenty posterior to the anus.

Diagnosis: Neoschöngastia carveri is the only member of the genus in which the sternal setae are arranged in rows rather than pairs, and the only species that has more than six sternal setae.

Nymph, Fig. 12

Body: Length 850, width at shoulders 370, width behind shoulders 350. The shoulders are indistinct in the single specimen available. The entire body is clothed with feathered setae.

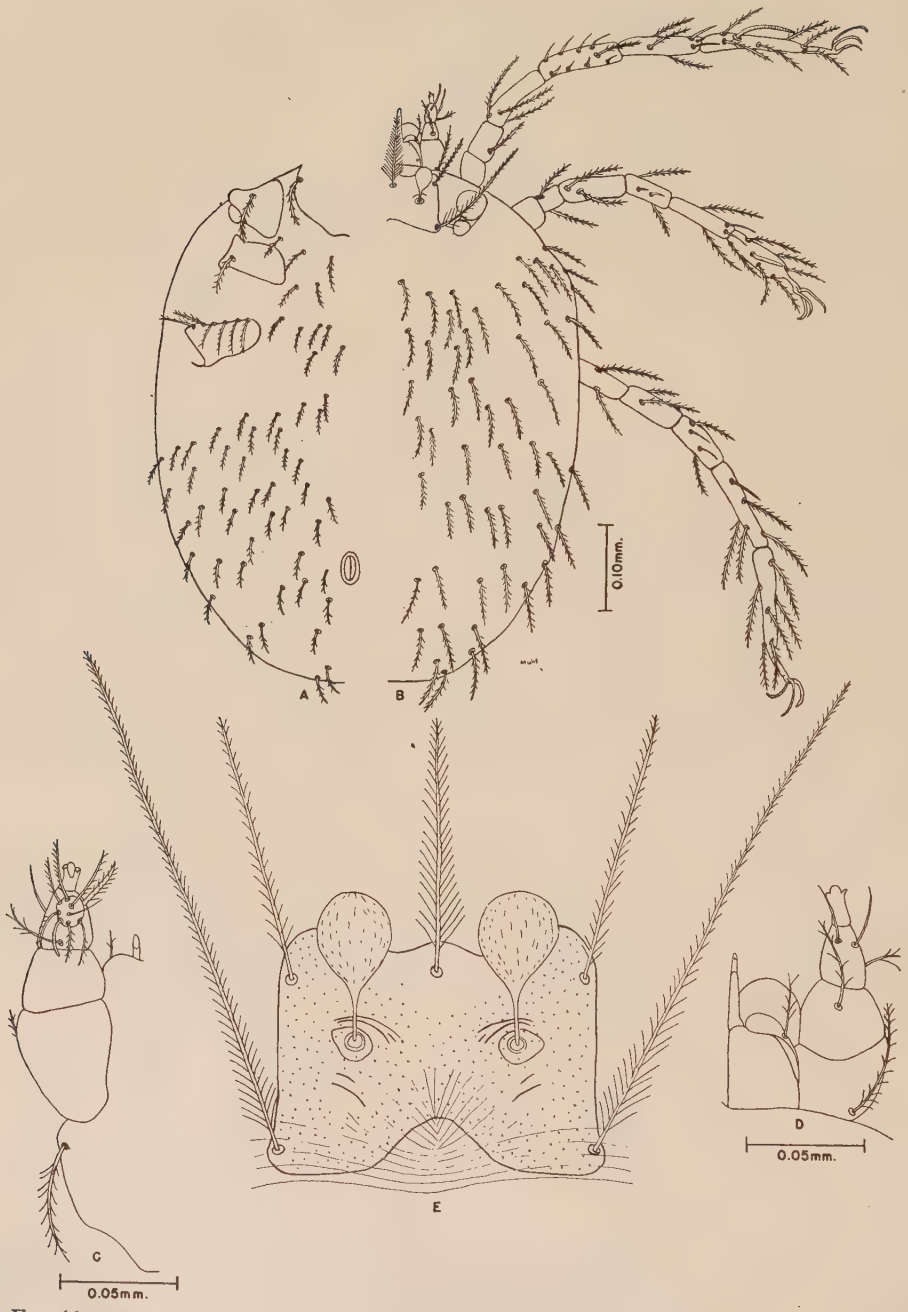

FIG. 11. *Neoschöngastia carveri* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

Because of the indistinct shoulders the body does not have the typical figure-eight appearance of other members of the family TROMBICULIDAE. The integument is striated between the depressed discs from which the body setae arise. There are no eyes. The genital opening is partially developed and is peculiar in that the anterior pair of genital suckers are elongated ovals while the posterior pair are more nearly circular in outline. The anal opening is slightly less than its own length posterior to the genital opening.

Gnathosoma: Segments one of the palps are fused with the maxillae and form a ventral sheath for the chelicerae. There are feathered setae over the ventral portion of this plate and a number of nude setae at its anterior margin. Palpal segment two originates from the anterior lateral area of one. It bears a total of fourteen or more feathered setae of which four are dorsal while the remainder are lateral. Palpal segment three bears a ring of eight feathered setae. Palpal segment four has a basal lateral pair of feathered setae between which is a single nude seta, another nude seta laterally just below the claw, a dorsal feathered seta, and a medial feathered seta at the posterior ventral angle. Medially and dorsally, there are two spines just proximal to

FIG. 12. Nymph of *Neoschöngastia carveri* n. sp. A, medial aspect of palp; B, leg I; C, lateral aspect of palp; D, dorsal setae; E, crista; F, genital and anal plates.

the claw. The claw is 30 long, curved, and sharp. The fifth palpal segment is distinctly truncate and is provided with six leaf-like setae on its anterior distal surface, one leaf-like seta at its posterior distal angle, a basal and a distal striated sensory seta on the lateral aspect, and four whip-like setae on the posterior aspect. The basal segments of the chelicerae are long while the distal chitinous segments are relatively short and bear about nine minute teeth along the dorsal edge. Epistome with a pair of feathered setae in tandem, anterior margin with seven teeth.

Legs: Leg I is longest, leg IV next, and legs II and III are shorter and sub-equal. Coxae clothed with feathered setae but less heavily than the adjacent body areas. Legs with striated sensory setae on genua, tibiae, and tarsi. The striated sensory setae on leg I are larger and more

numerous than on the other legs. A pair of claws on each tarsus with no empodium. Tarsus I; length 147, width 50. Tibia I, length 67.

Crista: Short with a single sensillary area. Sensillary area truncate posteriorly, strongly concave anteriorly. Pseudostigmata 37 from center to center; cuticle over area thrown into irregular folds or striae. Sensillae long and whip-like with a few minute spines at their base.

Setae: Setae on appendages unless otherwise noted are feathered but not so densely as those on the body. Body setae of three types. The anterior setae are finer than the posterior setae. Posterior setae arise from prominent papillae and are either long (50) or short (27). The shorter setae are more numerous than the longer.

Remarks.—Specimens of chiggers from a reef heron *Demigretta sacra* that was shot at Port Ajayan, Guam were put into a standard culture dish 17 July 1945. On 9 August 1945 one nymph was seen and one larval skin; these were mounted. The nymph is described above, the larval skin was identified as *Neoschöngastia carveri.*

COLLECTIONS OF *Neoschöngastia carveri* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. N. Strong	Agfayan Bay, Guam	*Numenius phaeopus*	4 June 1945	3
J. N. Strong	Port Ajayan, Guam	*Demigretta sacra*	16 July 1945	Type, nymph, + 7
J. N. Strong	Ylig Bay, Guam	*Pluvialis dominica*	19 July 1945	2
A. B. Hardcastle	Hianza Shima, Okinawa	*Gygis alba*	26 July 1945	2
L. A. Posekany	Hianza Shima, Okinawa	*Sterna douglaii*	26 July 1945	3
L. A. Posekany	Hianza Shima, Okinawa	*Monticola solitarius*	3 Aug. 1945	2
A. B. Hardcastle	Tsuwa Village, Okinawa	*Monticola solitarius*	9 Aug. 1945	1
L. A. Posekany	Yona Genina, Okinawa	*Monticola solitarius*	21 Aug. 1945	2
L. A. Posekany	Hentona, Okinawa	*Monticola solitarius*	23 Aug. 1945	4
C. A. Woodbury	Inarajan, Guam	*Numenius phaeopus*	2 Sept. 1945	2
C. A. Woodbury	Liguan Point, Guam	*Arenaria interpres*	21 Sept. 1945	1
J. N. Strong	Bulubul Is., Ulithi Atoll	*Pluvialis dominica*	21 Aug. 1945	1
J. N. Strong	Akarakoro Pt., Peleliu Is.	*Heteroscelus incanus*	6 Sept. 1945	1

Material: Type, other larvae, and the nymph have been deposited in the U. S. National Museum. Identified material will be shipped to the South Australian Museum, Adelaide, South Australia.

Discussion.—*N. carveri* is established on land birds on Okinawa but is apparently confined to shore birds on Guam, Ulithi, and Peleliu. This species is named for R. K. Carver who reared the nymph.

Neoschöngastia namrui n. sp.
Larva, Fig. 13

Body: Oval with indistinct shoulders. Length 474 to 625, width 260 to 390. Color orange. Striae moderate. Eyes exceptionally large, opposite scutum, anterior eyes the larger.

Gnathosoma: A long feathered seta with numerous barbs on palpal segment one. Palpal

FIG. 13. *Neoschöngastia namrui* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

segment two rounded, with dorsal feathered seta on its posterior lateral margin. Palpal segment three with a dorsal feathered seta with fewer and shorter barbs than on palpal segment two. Palpal segment four with feathered dorsal and ventral setae; lateral seta with at times a single barb. Palpal claw short, strongly curved, trifurcate with the central element the largest. Palpal segment five with one striated sensory seta, one nude seta, and seven feathered setae of which the dorsal is by far the largest. Galeal seta feathered. Basal segment of chelicera long, distal segment narrow with a blade-like apex, an anteriorly projecting dorsal tooth, and a recurved ventral tooth.

Legs: A long feathered seta on coxa I, a shorter feathered seta on coxa II, and from seven to twelve setae on the anterior margin of coxa III. Most specimens had the same number of setae on coxa III but one specimen had seven on one side and nine on the other. A striated sensory seta on tarsi I and II, no whip-like setae on tarsus III, nude setae on tibiae I, II, and III; and a concentration of nude setae on the dorsal surface of the genua of legs I, II, and III.

Scutum: Trapezoidal with the anterior width greater than the posterior. All margins sinuous. Stippling over the entire scutum. Horizontal parallel striae over the posterior two-fifths. Anterior median and posterior lateral setae feathered with long barbs. Anterior lateral setae feathered with shorter barbs. Sensillae capitate with spherical head beset with few setules and long, thin, tapered stalk. A hemispherical ridge just anterior to each pseudostigma. The standard data follow:

Guam specimens

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	69	53	38	36	42	46	60	72	77	32
	78	69	43	..	40	48	63	70	78	42
	77	63	40	36	41	46	63	73	73	42
	76	62	42	..	..	45	66	70	75	38
	74	60	39	38	39	48	61	70	70	36
Mean	75	60	40	37	40	47	63	71	75	38

Ulithi specimen

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
75	60	39	27	39	45	54	69	60	44

Okinawa specimen

AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
72	54	39	35	39	45	60	69	60	24

Setae: Dorsal setae feathered 44 long anteriorly, 32 long posteriorly. About 15 humeral setae on each shoulder and about 175 setae medial and posterior to them. Ventral setae similar to dorsal setae but shorter except for the two pairs of sternal setae. About one hundred and fifty setae posterior to the coxae of which approximately one-third are posterior to the anus.

Diagnōsis: Neoschöngastia namrui is close to *N. carveri* but differs from it in a number of characters among which are the following possessed by *N. namrui:* only four sternal setae, and AW greater than PW. In fact, no other previously described species in the Pacific area has an AW greater than PW.

Collections of *Neoschöngastia namrui* n. sp.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. M. Fritts	Ritidian Pt., Guam	*Numenius phaeopus*	29 May 1945	1
A. B. Hardcastle	Hianza Shima, Okinawa	*Gygis alba*	26 July 1945	1
C. A. Woodbury	Facpi Pt., Guam	*Heteroscelus incanus*	28 Sept. 1945	3
C. A. Woodbury	Facpi Pt., Guam	*Pluvialis dominica*	28 Sept. 1945	Type + 2

Material: Type and other material have been deposited in the U. S. National Museum. Identified specimens will be sent to the South Australian Museum, Adelaide, South Australia.

Discussion.—Except for the shorter scutal setae on specimens from Ulithi and Okinawa the Guam specimens appear to be identical with the others. *Neoschöngastia namrui* has been found only on shore-birds and specimens are probably carried from one island to another with regularity so that even such widely separated

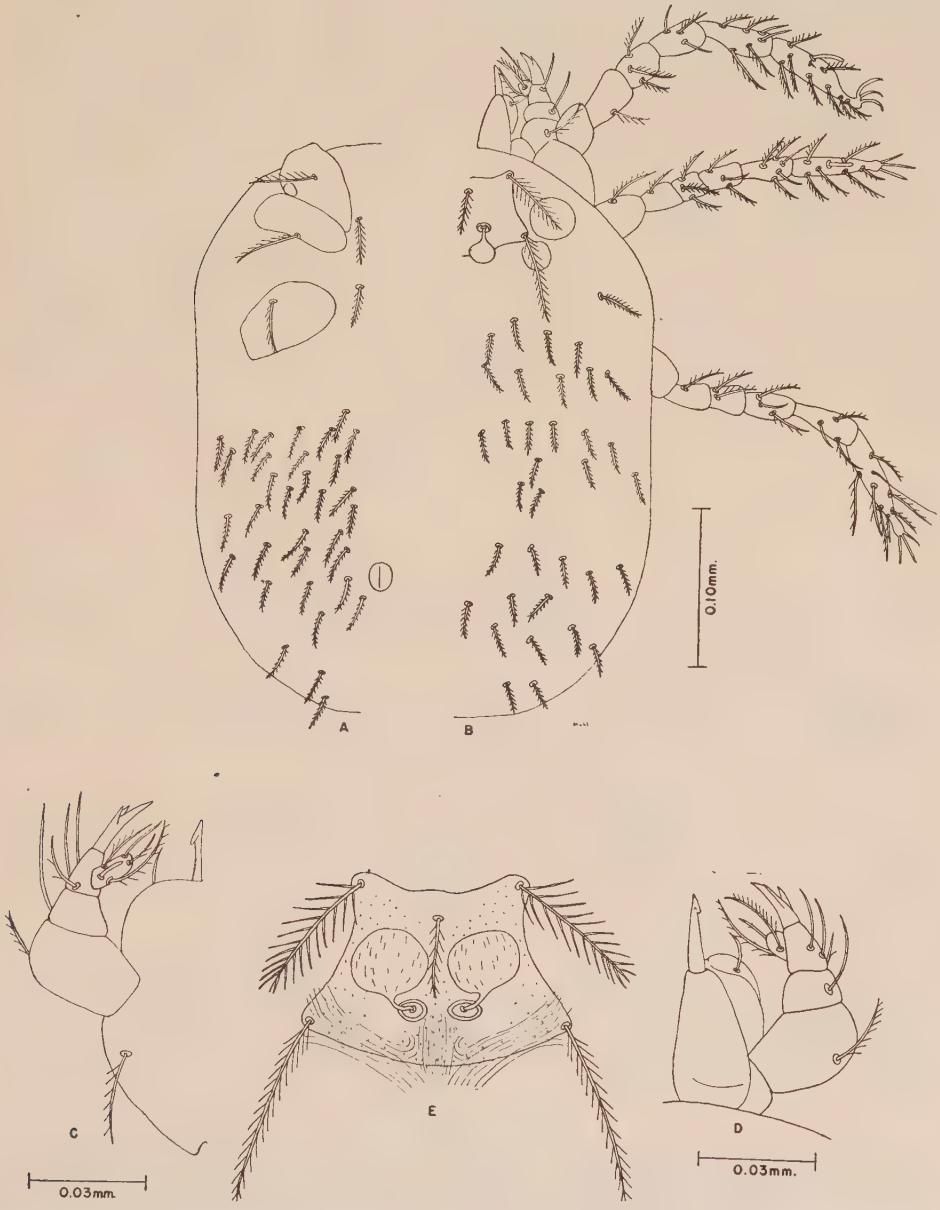

FIG. 14. *Neoschöngastia strongi* n. sp. A, venter; B, dorsum; C, ventral view of gnathosoma; D, dorsal view of gnathosoma; E, scutum. ×500.

populations as those on Okinawa and Ulithi may be in reality no more isolated from each other than are chiggers that parasitize rodents from one valley to another. This species is named after Namru, the naval abbreviation of Naval Medical Research Unit.

Neoschöngastia strongi n. sp.
Larva, Fig. 14

Body: Length 430 to 480, width 220 to 256. Oval with distinct shoulders. Eyes extremely large, anterior eye the larger. Striae strong.

Gnathosoma: Feathered seta on palpal segment one, a deep notch at the posterior medial junction of right and left maxillae. Palpal segment two with a feathered seta on which there are a few short barbs. Seta on palpal segment three stout and apparently nude but on one side of one specimen a fine barb was seen. Dorsal and lateral setae on palpal segment four nude, ventral seta with two or three fine barbs. Palpal claw trifurcate, the median element the stoutest. Fifth palpal segment with striated sensory seta at its base, a nude seta at the apex, and at least five feathered setae. Galeal hair nude. Basal segment of chelicera elongate, distal segment with blade-like apex, a small sharp anteriorly projecting dorsal tooth and large laterally expanded recurved ventral tooth.

Legs: A single seta on each coxa. Striated sensory setae on tarsi I and II. A whip-like seta on tarsus III. Nude setae on genu, tibia, and tarsus I; genua and tibiae II and III.

Scutum: Irregular in shape, anterior margin sinuous, lateral margin concave, posterior margin convex, light stippling over entire scutum. Faint striae over posterior margins that radiate from a point posterior to the scutum. Scutal setae feathered, barbs on anterior median seta the shortest, pseudostigmatic organs fit in a depression anterior to the pseudostigmata. Sensillae capitate with heads circular in outline and covered by fine setules. The standard data follow:

	AW	*PW*	*SB*	*ASB*	*PSB*	*AP*	*AM*	*AL*	*PL*	*S*
	39	66	15	33	15	34	27	39	45	21
	39	63	11	33	13	33	24	36	36	22
	42	67	13	35	15	36	22	35	35	..
Mean	40	65	13	34	14	35	24	37	39	22

Setae: Dorsal setae 17 to 35 long, the longest setae are the anterior ones. A pair of humeral setae plus about 70 posterior setae arranged in irregular rows. Ventral setae similar to the dorsal setae, two pairs of sternal setae plus about 64 posterior setae. About twelve setae posterior to the anus.

Diagnosis: The stout nude seta on palpal segment 3 is unique. In addition to this character the proximity of the pseudostigmata and the depressions in the scutum for the sensillae anterior to the pseudostigmata and the arrangement of the striae are all distinctive.

COLLECTIONS OF *Neoschöngastia strongi* N. SP.

Collector	*Locality*	*Host*	*Date*	*No. of slides*
J. N. Strong	Piti, Guam	*Numenius phaeopus*	26 July 1945	1
J. N. Strong	Akarakoro Pt., Peleliu	*Arenaria interpres*	8 Sept. 1945	Type + 1

Material: Type and other material have been deposited in the U. S. National Museum.

Discussion.—The limited material available shows few differences between the Guam and Peleliu populations. This species is named for J. N. Strong, its collector.

Neoschöngastia sp.
Nymph, Fig. 15

Body: Length 670 to 710, width at shoulders 300 to 390, maximum width behind shoulders 340 to 390. The posterior width is greater than the humeral width except in the widest specimen in which they are equal. The shoulders are prominent and separated from the posterior portion of the body by a deep cleft. The entire body is clothed with feathered setae. In appearance this nymph is typical of the family TROMBICULIDAE. The integument is paved with depressed discs posteriorly, from the center of which the papillae which bear the setae arise. Between the pits the cuticle is ornamented by wavy, delicate striae. There are no eyes. The genital opening is partially developed and two pairs of genital suckers are clearly visible in the posterior genital region.

Gnathosoma: Segments one of the palps are fused with the maxillae and form a ventral sheath for the chelicerae. Laterally and medially this plate is provided with feathered setae. There are five nude setae across its anterior margin. Palp segment two originates laterally from the anterior region of one, it bears three feathered setae, two of which are dorsal and one lateral. Segment three has a circle of seven feathered setae about its middle. Fourth palpal segment with one nude and three feathered setae on the lateral side, and one feathered seta dorsally. The three feathered setae are near the proximal margin, the nude seta is at the apex. Two large spurs proximal to the palpal claw and a single feathered seta in the proximal ventral angle are on the medial aspect of segment four. Fifth palpal segment or palpal thumb with five feathered, two nude setae, and one striated sensory seta laterally, six feathered and a single striated

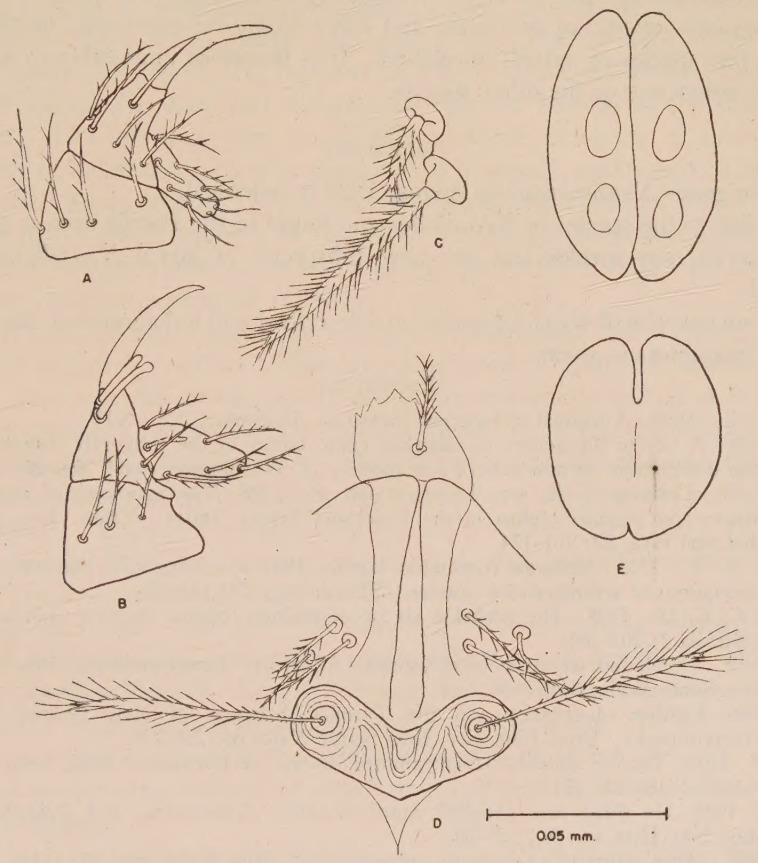

FIG. 15. Nymph of *Neoschöngastia* sp. A, lateral aspect of palp; B, medial aspect of palp; C, dorsal setae; D, crista; E, genital and anal plates.

sensory seta medially. Palpal claw narrow and 33 to 37 long. Chelicerae small, basal segment rounded, distal chitinous segment with a row of about eight minute dorsal teeth. Epistome with a single feathered seta and five teeth on its anterior margin.

Legs: All legs shorter than body. Leg I longest, IV shorter than I but longer than II and III which are sub-equal. Coxae sparsely clothed with feathered setae. Inconspicuous striated sensory setae on legs II, III, and IV; numerous striated sensory setae on the genu, tibia, and tarsus of leg I. Claws two on each tarsus, no empodium. Tarsus I, length 120 to 133, width 50 to 60. Tibia I, length 77 to 86.

Crista: Short with a single sensillary area. Sensillary area reniform with the hilus directed anteriorly. Pseudostigmata 37 to 43 from center to center. Cuticle ornamented with fine striae over the entire sensillary area. Sensillae are slightly clavate and are provided with short delicate spines along their entire length.

Setae: Setae on the appendages except as otherwise noted above delicate and feathered. On the body there are three types of setae. On the propodosoma the feathered setae have fine

branches and arise from simple discs. On the opisthosoma there are two stout types of setae one 50 to 70 long the other 20 to 23 long. Both types are thickly beset with short spines and arise from short papillae in the center of depressed discs. The shorter setae are slightly more numerous than the longer ones.

Remarks.—On 16 June 1945 red and yellow chiggers from a rock thrush, *Monticola solitarius,* were placed in glass vials (procaine tubes) with moist sand on Okinawa. The vials were sealed with rubber stoppers and flown to Guam. Upon arrival, contents of the vials were placed in standard culture dishes. The first nymph was seen on 2 July 1945. Unfortunately there were two species of chiggers, *Neoschöngastia americana solomonis* and *Neoschöngastia monticola,* on the thrush, and only one species of nymph developed. It is therefore impossible to assign this nymph to either one or the other species.

SUMMARY

1. The genus *Neoschöngastia* Ewing, 1929 is redefined.

2. A key to the species of *Neoschöngastia* found in the Pacific area is given.

3. Eleven new species and one new subspecies of larval *Neoschöngastia* are described.

4. Two nymphs of *Neoschöngastia* are described and a diagnosis of the genus on nymphal characters is given.

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IN MEMORIAM

Henry Baldwin Ward (1865–1945)

Henry Baldwin Ward died at Urbana, Ill., on November 30, 1945. Dr. Ward was born in Troy, N. Y., on March 4, 1865. He received the A.B. degree at Williams College in 1885 and, from 1888 to 1890, studied at the Universities of Göttingen, Freiburg, and Leipzig. At Leipzig he worked in the zoological laboratory of Rudolph Leuckart. He received the Ph.D. degree at Harvard University in 1892 and during the same year became instructor in zoology at the University of Michigan. The following year he became associate professor of zoology at the University of Nebraska, later was promoted to professor, and finally to head of the department. From 1899 to 1909 he served as dean of the medical school. In 1909 he was called to the University of Illinois to become professor and head of the department of zoology, a position he held until his retirement in 1933. He was married to Harriet Blair on September 11, 1894.

Dr. Ward's scientific career, extending over more than half a century, was one of intense and tireless effort embracing an unusual diversity of interests and activities. As a member of the faculty of a great university, and head of one of its major departments, he assumed the many administrative responsibilities that such a position entails. He was elected to offices in numerous scientific societies and rendered outstanding service in shaping the policies and assuring the success of these organizations. Special mention should be made of his achievements as an editor. As may be seen from the statement on its cover, this Journal was founded by Dr. Ward. The first number appeared in September, 1914, and it was issued under his editorship until 1932. While at the University of Nebraska he edited the Studies from the Zoological Laboratory and the publications of Sigma Xi including the *Quarter Century Record*. Largely through his instigation the *Illinois Biological Monographs* became available as mediums for publication. In 1917 *Freshwater Biology* was published under the joint authorship of Ward and Whipple. This ambitious undertaking resulted in a book that has been of great service to students of aquatic biology.

While at the University of Michigan, Dr. Ward began studying the biology of the Great Lakes region especially as it affected the fishes of these waters. In 1906 his summer activities were shifted to the west coast of the United States and Alaska where he began investigations that were continued, intermittently, for many years until he became recognized as an authority on the salmon of the Pacific.

In spite of these and other activities and responsibilities, to which must be added his teaching schedule for undergraduate students, Dr. Ward found time to continue his personal research and writing. In the *Index Catalogue of Medical and Veterinary Zoology* are found 136 entries for books, papers, and short articles of which he is author or co-author. The papers are devoted largely, though not exclusively, to recording the results of research in parasitology and they are the source of information that has been of great importance in furthering our knowledge of this subject.

Probably Dr. Ward's greatest ambition was to establish in the United States a graduate school of parasitology. One may surmise that he secretly hoped this school

might eventually acquire a reputation for high standards equal to that of Leuckart's at the University of Leipzig. All will agree, I believe, that this ambition was fully realized. From the time his graduate school was established at the University of Illinois in 1909 until his retirement in 1933, graduate students in parasitology were continually trained under Dr. Ward's able tutorship. These students were in demand, many filling important and influential positions in educational and research institutions. Through the outstanding success of his graduate school, Dr. Ward succeeded in promoting study and research in parasitology to an extent that has few parallels in the annals of American education.—JESSE R. CHRISTIE, *Division of Nematology, U. S. Plant Industry Station, Beltsville, Md.*